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Fungi That Attack Microscopic Animals.
C. L. DUDDINGTON 377

FUTURE CONTENTS
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THE BOTANICAL REVIEW

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FUNGI THAT ATTACK MICROSCOPIC ANIMALS [1]

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INTRODUCTION

The term " predacious fungus " is usually applied to those microfungi that capture, kill and consume microscopic animals. Research during the last two decades has shown that this unusual mode of life is far from being uncommon, since the predacious fungi are to be found with great regularity in leaf-mould, rotting wood, soil, dung and decaying plant material of many kinds. In spite of their common occurrence and apparently world-wide distribution, however, they have attracted but little attention from botanists in general, and are scarcely more familiar to many students of fungi.

The means adopted by these fungi for capturing their prey are varied, but the most usual adaptation to the predacious life is the production of a sticky substance, by means of which an animal coming into contact with the mycelium of the fungus is held, as a bird is caught by bird-lime. In addition to the possession of this

[1] Some of the material contained here has been used in a thesis for the degree of Ph.D. in the University of London.

sticky fluid, there may or may not be structural modification of the mycelium to assist in the capture of prey. Mechanical traps, working on the principle of the rabbit snare, are also common. The animals captured are usually Protozoa or nematode worms, though several fungi are known to attack rotifers, and one species, *Arthrobotrys entomopaga,* has been observed (54) to capture springtails.

In addition to the actively predacious species which trap living prey, many fungi are known which live as internal parasites in amoebae, nematodes and other small animals, often causing severe epizoötics in Petri dish cultures. Most of these fungi have spores that stick to the exterior of the host animal on contact and germinate to produce an endozoic mycelium. These parasites, though not actively predacious, are usually classed with the predacious fungi, and an absolute distinction between internally parasitic and truly predacious habits is not always an easy one to draw, as may be seen, for example, in the genus *Nematoctonus.* Probably those species which have adhesive spores are best regarded as being predacious during their reproductive stage, the more so as their spores are often presented in such a way as to ensure the maximum probability of their being accidentally picked up by a passing animal.

HISTORICAL OUTLINE

The earliest record of a predacious fungus is the description of *Harposporium anguillulae* as long ago as 1874 (107). It was not until some 14 years later that Zopf, in a classical paper, recorded the first observation of the capture of living nematodes by *Arthrobotrys oligospora,* one of the commonest of all the predacious fungi (131). This fungus was first described as a saprophyte in 1852, but its predacious habit was unsuspected by its discoverer (84). Nearly 20 years later it was observed (129) that germinating spores of *A. oligospora* often produced a mycelium in which curving, anastomosing hyphae formed a complex system of loops, but the significance of this phenomenon was not realised. Zopf grew the fungus in Geissler chambers in company with nematodes, and was able to demonstrate that eelworms were caught by the loops, and that, after the death of the animal, which took place in from two to two and a half hours after capture, the fungus intruded a system of absorptive hyphae into the body of the nematode and

consumed its contents. Zopf regarded the capture of the nematode as the result of purely mechanical entanglement in the loops, which he thought to be elastic; the fact that a sticky substance was secreted was not recorded until later (40).

The history of *A. oligospora* is paralleled by that of *Dactylella ellipsospora.* This fungus was first described in 1851 as *Menispora ellipsospora,* and later transferred to the genus *Dactylella* after it had again been found growing on rotten wood near Birmingham, England (80). Fifty years later in America, Drechsler (40) demonstrated that a fungus whose conidia resembled those of *D. ellipsospora* was able to capture nematodes by means of adhesive knobs which were borne laterally on short stalks at frequent intervals along the hyphae. It is interesting to record that a fungus bearing similar knobs was recorded in 1888 by Zopf (131), but he did not see the conidia, and thought that the stalked knobs were spores.

The next discovery of importance was *Zoophagus insidians,* an aquatic Phycomycete which captures rotifers (125). This interesting fungus has since been observed by other workers in Britain (4) and elsewhere. The systematic position of *Z. insidians* is open to doubt. The earlier workers described sexual organs which appeared to place it in the Oomycetes, but there appears to be some possibility that there was confusion with another fungus, and it has been suggested (55) that *Z. insidians* might be better placed in the Zoopagaceae, a family of the Zygomycetes.

Some early records from France (116) are of historical interest. Parasites of amoebae were described and placed by their author, probably incorrectly, in the Saprolegniaceae. From the published descriptions and drawings it seems possible that we have here a very early record of the Zoopagaceae, for some of the structures figured and described are suggestive of *Endocochlus or Cochlonema.*

What might well be termed the modern era of the study of predacious fungi began in America in 1933 when Drechsler, in four short papers (27–30), announced the discovery of several entirely new kinds of predacious activity. This was followed by a long series of descriptions of new predacious species. His first communications were mainly concerned with the Zoopagaceae, a family consisting entirely of obligate predators, mostly attacking amoebae

in decomposing vegetable matter. In 1937 he published a long paper (40) dealing with the predacious hyphomycetes, in which, besides giving details of the predacious activity of *Arthrobotrys oligospora, A. superba, Dactylaria candida* and *Dactylella ellipsospora,* he described a number of new species belonging to the same predacious series. Since that time the predacious fungi have been particularly associated with his name, and the monumental store of information that he has unearthed and is continuing to uncover will stand as one of the classic researches of mycology.

In addition to the work of Drechsler, no historical account of the predacious fungi would be complete without reference to the interesting contributions which have recently come from France, particularly in the spheres of physiology and the possible use of predacious hyphomycetes for the biological control of nematodes parasitic in plants and animals. Reference to this work will be made later.

SCOPE OF THE PRESENT WORK

In a review of even so limited a field as that presented by the predacious fungi, a certain amount of selection is inevitable if the work is to be kept within reasonable length. In the present contribution consideration is given only to the two main groups of predacious fungi, namely, the Zoopagaceae and the predacious hyphomycetes, together with a few other forms which are included on account of their similar mode of life or because they are intrinsically interesting. No mention is made of the entomogenous fungi, with which the name of T. Petch will always be associated, nor of the various species of Chytridiales that have from time to time been described as parasitic on animals. Fungi parasitic on eggs of animals or on nematode cysts are not included, and species of doubtful validity are ignored.

In describing the predacious fungi, it will be convenient to consider them under four heads—the Zoopagaceae, the Arthrobotrys [2] series of predacious hyphomycetes, the endozoic predacious hyphomycetes, and other forms. In group four are included three aquatic species of doubtful systematic position, and some endozoic parasites mostly belonging to the Lagenidiales: the former because

[2] It must be understood that the use of this term here is one of convenience only, and that no taxonomic or phylogenetic implications are intended.

of the possible relationship of two of them with the Zoopagaceae, and the latter as examples of fungi outside the main predacious series that have adopted this unusual mode of life.

SYSTEMATIC REVIEW OF THE PREDACIOUS FUNGI

ZOOPAGACEAE. The Zoopagaceae are a family of Zygomycetes consisting entirely of predacious species, defined by Drechsler in 1938 (42). Most of them attack terricolous amoebae, but other hosts are recorded, and there are a few species, such as *Stylopage hadra,* that are sufficiently robust to capture nematodes. Their relationships are obscure; they have been included, not very comfortably, in the Entomophthorales by some authors and in the Mucorales by others; there is little doubt, however, that they should be given ordinal rank, and this has already been done by one author (5). Apart from their exclusively predacious habit, one feature that sets them apart from most other Zygomycetes is that their asexual spores appear to be true conidia and are not violently discharged. The conidia may be carried on relatively long aerial conidiophores, or nearly sessile on short sterigmata; they may be single, several along the length of the conidiophore, in groups or in chains. No indication of a double wall is observable under the most rigorous microscopical observation, nor does their mode of development suggest that they may be modified one-spored sporangioles; if they have been derived from some mucoralean ancestor with elongated sporangia, as are found in the Piptocephalidaceae, they have advanced so far along the evolutionary road that all trace of their origin has been lost.

Two forms of predacious activity are found in the family. In some, for example, *Cochlonema* and *Endocochlus,* the mycelium is thalloid and internally parasitic within the protozoan host, while in others (*Stylopage, Acaulopage* etc.) a well-developed filamentous mycelium is present, the prey being apparently captured by adhesion and its contents absorbed by a haustorial system of lateral branches. The genus *Bdellospora* is an interesting intermediate form, the thallus being developed outside the host amoeba by swelling of the infecting conidium, while nourishment is drawn from the host by means of haustoria as in the mycelial genera.

The family at present contains nine genera and 65 species, and has a wide distribution, members having been recorded from Eu-

rope, North America and Australia. There is little doubt that many more species await discovery.

The endozoic habit in the Zoopagaceae is well illustrated by the genus *Cochlonema*. In *C. verrucosum* (32, 72) infection of an amoeba is initiated by one of the fusiform conidia of the fungus becoming attached to the ectoplasm of the animal. A very fine germ tube is put out which penetrates into the endoplasm and gives rise at its apex to a globular swelling. This swelling becomes detached from the germ tube which, with the empty conidium, is cast away by the animal. The globular mass remaining in the animal swells, and from being subspherical it elongates to become sausage-shaped. As growth continues, it bends sharply over on itself and becomes coiled in a close spiral of about one and a half turns. In a mature thallus the diameter of an individual "hypha" is about six microns. As the fungus grows, the endoplasm of the amoeba is progressively absorbed.

During early stages of infection the amoeba continues to move about, ingesting food material and seeming little if at all the worse for the presence of the fungus. Multiple infection by more than one conidium is not uncommon, and living amoebae may be seen, each with several thalli at different stages of development, feeding on its endoplasm. Eventually, however, the effects of the fungus make themselves felt; as the endoplasm of the amoeba becomes more and more depleted, the animal becomes increasingly sluggish, finally assuming a more or less rounded shape and ceasing to move at all. The fungus continues to feed on the dead or moribund host until nothing but the shrivelled remains of the ectoplasm is left.

Reproduction usually begins when much of the endoplasm of the host has been consumed and the animal is practically, if not quite, dead. From various points on the coiled thallus delicate fertile hyphae grow out through the ectoplasm of the host and, turning upwards, rise vertically into the air. They become constricted at intervals and form chains of spindle-shaped conidia, 30 or more to a chain. When mature, the chains are easily disrupted, the individual conidia then being strewn about the surface of the substratum in large numbers where they can be picked up casually by passing amoebae, so that it is hardly surprising that in Petri dish cultures severe epizoötics among the amoebae may result.

Sexual reproduction occurs in the manner usual among Zygomy-

cetes. Slender outgrowths from the thalli emerge from the remains of the host, bearing club-shaped gametangia at their ends; the junction between suspensor and gametangium is often marked by an abrupt knee-shaped bend. After fusion of the gametangia, a zygospore develops in the usual way.

Various other species of *Cochlonema* have been described (32, 39, 43, 44, 47, 49, 55, 57, 70) from America, differing in size and shape of the thalli and spores. Most of them attack amoebae, but a few are parasitic in other Protozoa.

The genus *Endocochlus* resembles *Cochlonema* in habit, and is also parasitic in Protozoa. It is distinguished by the conidia being borne singly, erect and practically sessile, as lateral outgrowths from prostrate fertile hyphae. Four species have been described, all from America (32, 37, 65).

An interesting intermediate stage between internal parasitism and the strictly predacious habit is seen in *Bdellospora helicoides* (32). The conidia in this monotypic genus are spindle-shaped and formed in erect chains as in *Cochlonema*. A conidium adheres to the exterior of an amoeba and intrudes a haustorium into its endoplasm. Instead of an endozoic thallus being formed, the conidium itself grows as nutriment is absorbed from the host, and develops into a lemon-shaped swollen body which is carried about by the amoeba until exhaustion of the endoplasm makes further movement impossible. At maturity, after the host has succumbed to the parasite, chains of conidia are formed, thus spreading the fungus among the population of amoebae. Sexual reproduction takes place by fusion of gametangia, the suspensors coiling tightly round one another. This unusual fungus has so far been recorded only in America.

Recently (70) some rather unusual zoopagaceous parasites of amoebae have been described from America. In *Aplectosoma* the endozoic thallus is cushion-shaped, and the conidia are formed in chains as in *Cochlonema*. Another unnamed amoeba parasite has a U-shaped thallus which bears spore-like bodies projecting to the exterior through the ectoplasm of the host. The relationship of these curious fungi to the rest of the Zoopagaceae is obscure, though it can hardly be doubted that they must be placed in that family.

As an example of the strictly predacious series of Zoopagaceae,

Stylopage rhynchospora may be quoted. This rather common fungus has been reported from America (44) and England (75). Instead of an endozoic thallus, we have here a well-developed mycelium of delicate non-septate hyphae which branch sparingly. Amoebae coming into contact with the mycelium stick to it, possibly on account of some adhesive substance secreted by the fungus. The ectoplasm of the amoeba is penetrated by a system of fine haustorial branches which originate at a point on the hypha in contact with the animal and spread throughout the endoplasm like the branches of a bush. By this means the fungus gradually absorbs the contents of its prey, so that eventually only the shrivelled remains of the ectoplasm remain attached to the mycelium. During the initial stages of attack the amoeba appears to be little harmed, as cytoplasmic streamings continue and the contractile vacuole functions normally. With further development of the haustoria inside it, however, the animal assumes a rounded shape, all movements cease and, with progressive absorption of the endoplasm, death supervenes.

The conidia of *S. rhynchospora* are borne singly at the apices of tall erect conidiophores. The spores are ovoid with a bluntly rounded beak at the distal end; this beak may be devoid of cytoplasm and may carry a certain amount of sticky fluid which sometimes causes conidia lying on the substratum to adhere together tip to tip. Sexual reproduction occurs by fusion of slender gametangia, and the resulting zygospore has a thick wall which, as is usual in the family, is ornamented with hemispherical warty protuberances.

A number of other species of *Stylopage* have been described (33, 34, 36, 37, 42, 44, 55, 62, 64, 81). Most of them capture Protozoa, but two species are large enough to prey on nematodes. *Stylopage hadra* (34) has a branched mycelium with hyphae about five microns wide. On contact with a wandering nematode, an intensely stickly fluid is secreted which holds the eelworm fast, in spite of its desperate struggles to get away. The chemical nature of this adhesive substance is unknown; it appears to be formed only on contact with a nematode and is extremely efficient, for, although the animal may pull the mycelium that holds it violently from side to side during its struggles, it is unable to escape once it is fairly held. When the prey has become quiescent, either from exhaustion or from some unknown other cause, a

bulbous swelling is formed on the fungal hypha at the point of contact. This swelling appears to act as an appressorium, and from it an outgrowth penetrates the integument of the nematode and becomes a starting point from which trophic hyphae ramify throughout the carcass of the animal and absorb its body contents. In the end, only the integument of the eelworm is left, and the protoplasm of the trophic hyphae is resorbed by the parent mycelium. *Stylopage leiohypha* captures nematodes in a similar manner; it differs from *S. hadra* in its smaller conidia and in the absence of the bulbous appressorium (36). Other members of the Zoopagaceae which capture eelworms in a like manner are *Cystopage lateralis* (47, 73) and *C. intercalaris* (55), in both of which the only known form of reproduction is by means of chlamydospores which are lateral in the first species and intercalary in the second. The curious species *Euryancale sacciospora* (44), internally parasitic in nematodes, is also worthy of mention here. Another unnamed species of *Euryancale* has recently been recorded by Drechsler (70).

The two remaining genera of Zoopagaceae are predacious on amoebae and other Protozoa, capturing their prey by adhesion to the mycelium. They differ from *Stylopage* in their habit of sporulation. In *Acaulopage* the conidia are borne singly at intervals along the hyphae, standing vertically on short sterigmata. A notable feature of this genus is the strong tendency for evacuation of cytoplasm from the distal end of the spore, leaving an empty appendage. Often the appendages are multiple; in *A. tetraceros* (32) the spore is shaped like an inverted cone, with from three to six filamentous appendages arising from its flattened distal end, while in *A. acanthospora* and related species the whole distal half of the spore is covered with short spiny appendages. The significance of these structures, if any, is unknown, but it is of some interest to note that several species of *Acaulopage* have been observed in aquatic or semi-aquatic habitats (113, 114), and it is not impossible that the appendages may be connected with floatation. A comparison is suggested with some of the aquatic hyphomycetes described by Ingold (89–93), though these do not appear to be predacious. Eighteen species of *Acaulopage* have been recorded, occurring in England (75, 77, 113, 114) and in America (33, 37, 42, 43, 47, 49, 55, 57, 62, 64).

Zoopage, the type genus of the family, also captures Protozoa,

principally amoebae. In this genus the conidia are elongated and formed in long erect chains attached basally to short sterigmata. The mode of predacious activity is similar to that in *Stylopage* and *Acaulopage*. The species of *Zoopage* are often difficult to identify, as the spore measurements and other diagnostic characters tend to vary slightly (77); it may be that the genus is somewhat labile and that a splitting of some of the species into varieties may become necessary in the future. The genus contains ten species (32, 37, 39, 42, 63).

Taken as a whole, the Zoopagaceae appear to form a compact and closely interrelated family. Their strictly predacious mode of life, the means adopted to snare their prey, their conidia and their very characteristic zygospores seem to mark them off sharply from the Mucorales, on the one hand, and the Entomophthorales, on the other, as a well-delimited group of Zygomycetes.

ARTHROBOTRYS SERIES OF PREDACIOUS HYPHOMYCETES. This is a group of genera belonging to the family Moniliaceae of the Fungi Imperfecti. Most of them attack nematodes and show various structural and physiological modifications to enable them to capture their living prey. These organs of capture may be classified into a number of types:

Adhesive Networks or Branches. In this type of mechanism, short lateral branches from the mycelium curl round and anastomose either with similar branches or with the parent hyphae, thus forming hyphal loops. From these, other loops arise, forming eventually complex systems of networks in three dimensions. Eelworms are captured by adhesion to the loops through the agency of a sticky secretion, and capture may be assisted by entanglement. This type of mechanism is found in *Arthrobotrys oligospora* (40), *Dactylella reticulata* (114), *Dactylaria thaumasia* (40), *Trichothecium flagrans* (74) and many others. Sometimes the networks are relatively simple, as in *Arthrobotrys musiformis* (40); the adhesive apparatus may consist of short branches which only occasionally fuse to form networks, as in *Dactylella cionopaga* (67), or of irregular lobed outgrowths from the mycelium, as in *Dactylella lobata* (79). In *D. gephyropaga* the networks are scalariform (40).

Adhesive Stalked Knobs. The predacious apparatus consists of a subspherical knob, borne on a short lateral branch from the

mycelium; the stalk is usually composed of one or two cells only, as in *Dactylella ellipsospora* (40) and *D. mammillata* (26). Nematodes are captured by adhesion to the knobs.

Non-Constricting Rings. Three curved cells join up to form a closed ring at the end of a short branch. A nematode accidentally passes its head into the ring in the course of its wanderings, and in attempting to force its way through, it becomes firmly wedged and is unable to get clear. No sticky substance or mechanical closure of the ring appears to be involved in this device. Usually non-constricting rings are accompanied by adhesive knobs, as in *Dactylaria candida* (40).

Constricting Rings. The constricting ring is built up of three curved cells, as in the previous mechanism, but here capture of the nematode is not passive. When a nematode pushes its head into the ring, the three cells suddenly enlarge to more than double their former diameter, at the same time shortening somewhat; the result of this is that the opening of the ring is practically obliterated and the nematode is held fast by constriction. The stimulus that operates this remarkable trap appears to be the friction set up by the nematode on the inner surfaces of the cells of the ring. Constricting rings are found in a number of species, for instance, *Arthrobotrys dactyloides* (40), *Dactylella bembicodes* (40), *Dactylaria gracilis* (77) and *Trichothecium polybrochum* (40).

Whatever the trapping mechanism, the ultimate fate of the prey is the same. When the animal is dead or moribund, its integument is penetrated by an outgrowth from the fungus, from which trophic hyphae grow and ramify throughout the carcass of the prey, absorbing its contents. Under favourable conditions, nematodes may be captured in very large numbers, especially by those fungi that employ the sticky network mechanism.

Unlike the Zoopagaceae, which appear to be obligate predators, members of the *Arthrobotrys* series can easily be isolated in pure culture, and grow well on most of the usual laboratory media.

To illustrate the working of adhesive networks, *Arthrobotrys oligospora,* the best-known of all the predacious fungi, may be chosen as a typical example. The mycelium consists of branched, septate hyphae which, in Petri dish cultures, spread in radiating lines over the surface of the medium. If nematodes are present in the culture, networks are formed freely, mostly on the surface

of the medium, though they may sometimes be submerged. A network is initiated by a short lateral branch curling back so that its tip comes into contact with its parent hypha a short distance away from its point of origin. Anastomosis occurs between the tip of the branch and the main hypha, forming a loop. Other loops are formed in a similar manner, on or near the original one, eventually forming complex networks, sometimes containing a dozen or more meshes. The hyphal elements forming the loops are noticeably wider than the subtending hyphae, and are also distinctly broader in the middle than at their extremities where they anastomose with other loops or with the main mycelium; in this respect they resemble the cells of the non-constricting and constricting rings, though whether this fact has any evolutionary significance, or whether it is purely functional, is hard to say.

A notable feature of the networks, and one that is very striking when viewed in situ under the microscope, is that the individual loops tend to be oriented in planes at right angles to one another. Some of the loops lie flat on the substratum on which the fungus is growing, while others stand erect like croquet hoops. The general effect is reminiscent of the semi-circular canals of the mammalian ear, and it is probable that this positioning increases their efficiency, for a nematode stuck to one loop may easily become entangled in others in the course of its efforts to free itself. The cells of the loops contain dense cytoplasm and a number of large vacuoles; they often stain rather deeply with haematoxylin or chlorazol black.

The sticky material that holds the prey appears to be secreted by the cells of the loops on contact with the nematode. Soon after capture, a quantity of viscous fluid can be seen in the region of the point of contact, and it seems clear that it is this fluid that is holding the animal. The action of the fluid is extremely efficient; although eelworms are active animals, and specimens more than half a millimetre long may be captured, they seldom escape, and the efficacy of the mechanism does not appear to be adversely affected by moisture.

Death of the nematode commonly occurs two hours or so after capture. The precise cause of death is somewhat in doubt; it is possible, though unlikely, that the animal may succumb to ex-

haustion after its violent struggling to escape. Production of a toxin by the fungus can not be discounted, though there is no evidence whatsoever in support of this. The theory that the animal is killed mechanically by intrusion of an outgrowth from the fungus (40) has, in my opinion, little to recommend it; although such an outgrowth is invariably formed, it usually does not appear until the animal is moribund. Further research on this point is badly needed.

When the nematode is dead or dying, its integument is penetrated by a short branch from the fungus, formed at the point at which the prey is held. Within the animal this branch swells to form a globose structure, the infection bulb, which may be anything from one half the diameter of the nematode upwards; where well developed, the bulb may completely span the animal's internal diameter. From this there grow out trophic hyphae which spread from the bulb in both directions and eventually fill the body of the eelworm. These trophic hyphae are septate, and at first they are filled with cytoplasm. They absorb the body contents of the eelworm, passing the food material so obtained back to the parent mycelium; as this work nears completion they become progressively emptied of protoplasm. Eventually only the integument of the eelworm remains, filled with the now empty trophic hyphae.

Arthrobotrys oligospora reproduces by means of two-celled pyriform conidia which are formed in "heads" at the apices of erect conidiophores. In older cultures the conidia may be in discrete whorls formed successively down the conidiophore; this tendency to nodal arrangement of the conidia is particularly noticeable in pure culture, where a single conidiophore may bear more than a dozen whorls of spores. The conidia themselves are carried on very short sterigmata. Each conidium is divided into two cells which are usually of unequal size, the distal cell being the larger, and there is usually a distinct "waist" between the cells. On germination the germ tube usually arises from the smaller proximal cell. The amount of sporulation varies according to conditions and is usually preceded by an initial feeding period, during which the young mycelium gorges itself on eelworms. Different isolates appear to vary in their intensity of sporulation and also in their aggressiveness towards nematodes; this is a phenomenon which calls for further investigation.

Arthrobotrys superba (9, 40) and *A. conoides* (40, 77) both resemble *A. oligospora* closely, differing mainly in the size and shape of their spores. The conidia of *A. conoides* are similar in shape to those of *A. oligospora,* differing in their greater length, while in *A. superba* the spores are smaller and straighter-sided. In both these species the tendency to nodal development of conidia is strongly marked. In *A. cladoles* (40), *A. cladodes* var. *macroides* (53) and *A. robusta* (80) a different habit of sporulation is found. Instead of the conidia being formed in whorls down the conidiophore, the fertile hyphae themselves are branched, a single head of conidia being formed at the apex of each branch. These three species all capture nematodes by means of adhesive networks, as in *A. oligospora.*

Arthrobotrys musiformis (40, 75) differs from the foregoing species in the relative simplicity of its traps. Instead of complex three-dimensional networks being formed, the traps often consist of single loops, and it is not uncommon to find a loop in the form of a ring composed of four cells. This arrangement is reminiscent of the non-constricting and constricting rings formed by other predacious hyphomycetes, except that the number of cells in the ring is one greater. Similar structures are sometimes formed by proliferation from the adhesive knobs in *Dactylella ellipsospora,* and it is tempting to speculate on a possible evolutionary connection between the condition in *D. ellipsospora* and the three-dimensional networks, on the one hand, and the non-constricting and constricting rings, on the other.

In *Arthrobotrys musiformis* the conidia are large, two-celled and somewhat curved. They are formed in a loosely capitate arrangement at the apex of an erect conidiophore, and in their general appearance are more suggestive of the genus *Dactylaria* than of *Arthrobotrys.* This approach to *Dactylaria* is even more pronounced in *Arthrobotrys dactyloides,* a species that captures nematodes by means of constricting rings.

The adhesive network is found in all the four principal genera of the *Arthrobotrys* series, and, if agar cultures are accepted as a criterion, it is probably the most efficient type of predacious apparatus. In an active culture the heaps of dead and dying eel-worms captured by the fungus may be visible to the naked eye, and under the microscope they may completely obscure the traps

that have caught them. Among genera having highly efficient networks may be mentioned *Dactylaria scaphoides* (115), *D. thaumasia* (40), *D. psychrophila* (53), *Dactylella reticulata* (114) and *Trichothecium flagrans* (74).

A different form of network is observed in *Dactylella gephyropaga* (40). Short lateral branches from the hyphae grow out at right angles, at approximately equal intervals. The tips of these branches are then connected to form scalariform networks in one plane. This species has so far been reported only from America.

Clearly related to the network is the type of trapping device that takes the form of adhesive branches. In *Dactylella cionopaga* (67, 75) short branches, usually one, two or three cells long, stand out at right angles to the hyphae that subtend them. These branches are sticky and serve as an efficient means for the capture of nematodes. Sometimes the branches curve over and anastomose to form loops, but the complex three-dimensional type of network is seldom formed. In young cultures *D. cionopaga* can easily be recognised by the spiky appearance of the hyphae with the branches in the one-celled stage. *D. cionopaga* appears to be a relatively common predacious fungus, both in America and England. It has also been observed several times in Denmark by Shepherd.

In *Dactylella lobata* (79) the predacious apparatus consists of one-celled lobes projecting from the hyphae at right angles. In the one-celled condition these lobes, which are subspherical to ovoid, recall the adhesive knob type of trap without the stalk, but they are subject to proliferation, similar lobes growing out from them to form a moniliform branch. By anastomosis simple networks may even be formed. The resemblance of this arrangement to the adhesive branches of *D. cionopaga* is manifest, and it also suggests comparison with the stalked knobs found, for instance, in *D. ellipsospora;* these resemblances may have some evolutionary significance, but the danger of drawing conclusions from evidence of this sort can not be too strongly emphasised. Nothing is at present known about the phylogeny of the predacious fungi, and such resemblances as have been pointed out above may well be homoplastic.

The stalked adhesive knob is known only in the genera *Dactylaria* and *Dactylella.* In *Dactylella ellipsospora* the knobs are sub-

spherical, slightly longer than they are broad, and attached to the mycelium by short stalks consisting of one or, more commonly, two cells. The knobs are formed at fairly regular intervals along the hyphae, and they are often arranged with the stalk standing vertically so that the knob is slightly raised above the surface of the substratum; this position probably assists the capture of surface-moving eelworms. On coming into contact with the knob, the nematode is held by a sticky substance, and in struggling to get away it often comes into contact with one or more other knobs and so is held more securely. When the animal has become quiescent, an outgrowth from the knob penetrates its integument and gives rise within it to a globular infection bulb, as previously described for *Arthrobotrys oligospora,* and from this bulb trophic hyphae grow out in the usual manner and absorb the contents of the eelworm. *Dactylella ellipsospora* reproduces by means of conidia which are formed singly at the apices of erect conidiophores; as in most species of *Dactylella,* the spores are diamond-shaped and multi-cellular, one of the central cells being very much larger than the rest.

Occasionally the adhesive knobs of *D. ellipsospora* show proliferation. From the knob a branch grows out which, curling round, rejoins with the knob, forming a loop of usually about four cells. The loops so formed are not unlike those of *Arthrobotrys musiformis.* Different strains of *D. ellipsospora* appear to vary in the strength of this tendency to proliferate, and in one strain that has recently come to my notice proliferation is the rule rather than the exception. At first sight, under the low power of the microscope, this proliferating strain could easily be mistaken for *Arthrobotrys musiformis.*

The stalked knob trap shows great uniformity among those species that possess it, differing only slightly in size and in the length of the stalk. *Dactylella mammillata* (26) differs from *D. ellipsospora* only in the shape of the conidia, with their pronounced mammillate tips. In *D. asthenopaga* the knobs are slightly smaller, and the conidia are fusiform, with the cells more or less all the same size; this delicate species captures rather smaller eelworms than the two foregoing.

Of the fungi that capture nematodes with the aid of non-constricting rings, *Dactylaria candida* (40) is the best known. The mycelium of septate hyphae bears stalked adhesive knobs in ad-

dition to the rings. Each ring is composed of three cells which are curved in such a way as to close up and form, by anastomosis, a ring. Where the tip of the (morphologically) distal cell joins the proximal one, the two cells are together fused with the stalk; this latter consists usually of two or three cells and is commonly somewhat longer and more slender than the stalk of the constricting type of ring described below.

The non-constricting ring appears to work purely by passive ensnarement. If a nematode casually pushes its head into the opening, the efforts of the animal to push its way through result in its becoming firmly wedged, since the internal diameter of the ring is somewhat less than that of the eelworm. There is no actual swelling or constricting of the ring, nor does any adhesive substance appear to be involved. Once caught, the eelworm is firmly held. As it struggles to free itself it not infrequently happens that the ring is torn away, and the eelworm escapes, still encircled. This may happen more than once, and in a flourishing agar culture of *D. candida* one may see eelworms moving about actively, wearing several rings as an indication of a number of escapes from capture. Such an escape is, however, only of short duration. Even from the detached ring, an outgrowth soon penetrates the integument of the unfortunate animal and, after it has been killed or rendered helpless, hyphae grow out from the cells of the ring and fill its body, eventually absorbing its contents. If the ring is still attached to the mycelium the material absorbed is passed back to the hypha that subtends the ring; where, however, an eelworm has been killed by a detached ring, a new mycelium grows out from its carcass, the ring thus serving as a means of vegetative reproduction. This phenomenon caused one observer (124) to mistake the rings of *D. candida* or a similar species for conidia, and to name the fungus *Annulosporium nematogenum.*

Hyphomycetes with non-constricting rings are usually provided also with stalked adhesive knobs, and there is some variation among the different species in the relative importance of the rings and knobs in the capture of prey. Thus on agar in Petri dish cultures *Dactylaria candida* captures most of its victims by means of the rings, the stalked knobs being seldom effective (40). This tendency is even more marked in *Dactylella leptospora* (40), where adhesive knobs may be formed on germinating conidia. *Dactylella lysipaga* also bears knobs that are sledom used in agar

cultures, most of the work of capturing nematodes being performed by the non-constricting rings with which this species is also provided (40). It must be pointed out, however, that we do not know whether this applies to the fungus growing in its natural habitat where totally different conditions may prevail. It is very dangerous to draw conclusions from agar cultures about the behaviour of fungi under natural conditions.

Of all the means by which predacious hyphomycetes capture their prey, the constricting ring has aroused the widest interest. This remarkable device was first fully described in 1937 by Drechsler (40) for *Dactylella bembicodes* and related forms. In *D. bembicodes* the rings are formed at intervals along the hyphae, each ring being carried on a short, stout, two-celled stalk. Initially the ring appears as a lateral branch which, as it grows out from the main hypha, curls round so that the distal end is brought into contact with the lower part. Anastomosis then takes place, the curved portion forming the ring and the lower part the stalk. When fully formed the ring consists of three curved cells, each of which is wider in the centre than at the ends; the outer side of each cell forms the arc of a circle, while the inner edge is nearly straight, so that while the profile of the ring is circular, the central opening is roughly in the form of a triangle. The stalk usually has two cells, the distal one of which is fused with two of the ring cells. The cells of the ring are somewhat thicker, and the stalk shorter, than in the non-constricting type of ring.

When *D. bembicodes* is growing on agar in the presence of nematodes, the rings are usually orientated perpendicularly to the surface of the medium, either just above or, very commonly, just below it. The rings are thus placed like rabbit snares so that wandering nematodes may readily pass their heads into them. When this happens the three cells of the ring quickly expand to more than twice their original diameters, gripping the nematode and severely constricting it beyond any hope of escape. The stimulus that produces the reaction appears to be a contact one due to the friction of the eelworm's body on the inner surfaces of the ring cells, for it has been shown (8) that rubbing the inside of the ring with a very fine glass micromanipulator needle produces a like result. The time taken for the cells to expand fully, once the reaction begins, is a matter of only a fraction of a second.

When an eelworm has been captured it struggles violently for a time and, since the rings are set fairly close together, it not uncommonly thrusts its tail into another trap and thus is doubly held. After a time it becomes quiescent, probably either through exhaustion or mechanical damage, and is then invaded by trophic hyphae which grow out of the cells of the ring and, penetrating the integument of the animal, extend throughout its body. Absorption of the body contents follows in the usual way. When the substance of the victim has been completely absorbed the trophic hyphae also lose their protoplasm.

The constricting ring trap is found in all the four principal genera of the Arthrobotrys series, and in most instances is remarkably uniform in structure. In *Arthrobotrys dactyloides, Dactylaria brochopage, D. gracilis, Trichothecium polybrochum* and a number of other species the rings follow closely the pattern of those in *Dactylella bembicodes.* Sometimes minor modifications of structure occur; thus in *Dactylella doedycoides* (45) there is a small median projection on the inner face of each cell of the ring, and this species also differs from most in having a slightly swollen tip to its conidiophore, though these features appear to be absent in at least one English strain (26). This median protrusion of the ring cells is to be noted also in *Dactylella heterospora* (51) and *D. acrochaeta* (71). A curious modification prevails in *D. coelobrocha* (61), where, owing to the peculiar formation of the ring, the captured nematode is held by two only of the swollen ring cells.

A much simpler organization is found in *Tridentaria implicans* which has been recorded as capturing nematodes already weakened by an internal parasite of unknown nature (45). The eelworms appear to stick to the tips of undifferentiated hyphal branches which, after capture is effected, often continue to grow, winding round the body of the captive as they do so. This species appears to be only weakly predacious, as is indicated by its habit of attacking eelworms that are already parasitised by another organism. *Triposporina aphanopaga* also captures nematodes by means of undifferentiated hyphal branches which are probably adhesive, though the presence of a sticky secretion does not appear to have been demonstrated with certainty (40).

Tridentaria carnivora, which captures the testaceous rhizopod

Difflugia constricta, is one of the few predacious hyphomycetes that prey on animals other than nematodes. Here again there are no specialised organs of capture; the rhizopods appear to stick to the hyphae of the fungus which thrusts a branch into the mouth of its victim, the branch then giving rise to a haustorial system within the animal (41). Other hyphomycetes preying on rhizopods are known. *Pedilospora dactylopaga* has short adhesive processes at intervals on its hyphae, by means of which it is able to capture individuals of *Difflugia globulosa* and *Trinema enchelys* (31). More nearly allied to the main series of predacious hyphomycetes is *Dactylella passalopaga* which craftily makes use of the feeding habits of the rhizopods on which it preys in order to bring about their undoing. The animal concerned is *Geococcus vulgaris,* a testaceous rhizopod which commonly feeds on *Pythium* oospores by applying its mouth to the oospore wall and sealing the contact with a yellow secretion which is apparently similar to the substance used to close up the test during encystment. When the protozoan is rash enough to apply this technique to filaments of *D. passalopaga,* the fungus responds by thrusting a lateral branch into the mouth of the animal, and the branch then expands to form a simple or lobed enlargement which is wider than the orifice. The animal is thus unable to withdraw, and its death and absorption by the fungus ensue (38). *Eughypha levis,* another testaceous rhizopod, is also captured by *D. passalopaga.* This piece of opportunism on the part of the fungus suggests the interesting possibility that the capture of amoebae by mycelial Zoopagaceae may be something of like nature, the initial attack coming from the amoeba, not from the fungus.

Dactylella tylopaga also captures Protozoa, the victims here being amoebae (35). The hyphae of the fungus bear rounded adhesive processes; when an amoeba has stuck to one of them, an extensive haustorial system of twisted hyphae is formed within the endoplasm, which is progressively absorbed. The unusual conidia of this species, with their empty appendages and short branched conidiophores, make its inclusion in the genus *Dactylella* open to doubt.

It is rather strange that in the *Arthrobotrys* series of predacious hyphomycetes only one species has been recorded as capturing arthropods. *Arthrobotrys entomopaga* is predacious on spring-

tails (*Collembola*) which it captures by means of short adhesive processes (54). This interesting species has so far been found only in America.

ENDOZOIC PREDACIOUS HYPHOMYCETES. The endozoic predacious hyphomycetes are a rather mixed group, having the common character that the main vegetative mycelium is within the body of the host, only the fertile hyphae being external. They attack nematodes, infection usually being brought about by means of adhesive spores which stick to the integument of the animal on casual contact. As the spores are commonly produced in large numbers, and as the host animals are numerous and gregarious, substantial epizoötics may ensue, at any rate in Petri dish cultures. Two of the commonest of the predacious fungi, *Harposporium anguillulae* (107) and *Acrostalagmus obovatus* (48), are included in this group. The conidia of the endozoic hyphomycetes are usually small, slimy phialospores being common. The genus *Nematoctonus,* with clamp connections on its fertile hyphae, is unique in this series; it is probably an imperfect basidiomyete.

Harposporium anguillulae is very common in soil and in decaying plant material of various kinds. Nematodes are infected by means of spores which stick to their integument. The spores are sickle-shaped, the proximal end being pointed and the distal end bluntly rounded; adhesion to the eelworm probably depends on the presence of a sticky substance, though it has been suggested (99) that the skin of the animal may be penetrated by the pointed end of the spore. Multiple infection is not unusual. On germination the spore produces a germ tube that penetrates the integument of the host and gives rise within it to a mycelium of sparingly branched septate hyphae. The mycelium continues to grow at the expense of the host which, after becoming increasingly sluggish in its movements, eventually dies. Finally the entire contents of the nematode are consumed by the parasite, leaving the integument filled with mycelium.

Sporulation occurs freely after the tissues of the animal have been used up. A lateral branch from the mycelium forms a pyriform swelling just beneath the integument of the host. From this an apical outgrowth penetrates the integument and grows out from it as a fertile hypha, consisting usually of from two to four cells, though exceptionally it may be longer. The fertile

hyphae are usually bluntly rounded at the distal end and somewhat constricted at the proximal end where they issue through the integument of the animal, and they bear laterally, singly or in groups, subspherical phialides, each with a short, slender neck, which may be somewhat curved. Each phialide produces in succession usually from one to five spores which are elongated and sickle-shaped. Many fertile hyphae are formed, and the conidia are very numerous, littering the surface of the substratum. In agar cultures, epizoötics due to *H. anguillulae* often persist for a long time, usually being terminated by the drying up of the culture.

In addition to the fertile hyphae bearing conidia, the mycelium within the host sometimes forms chlamydospores. These are somewhat swollen, thick-walled cells, formed sometimes singly, but much more commonly in rows of three to six or more, by the swelling of cells of the vegetative mycelium and deposition within them of abundant cytoplasm, followed by thickening of the cell-walls. Sometimes chlamydospores are formed outside the body of the host on short lateral branches of the mycelium similar to those on which the fertile hyphae originate. Under moist conditions these chlamydospores germinate, producing fertile hyphae bearing phialides and spores. The formation of spores in *H. anguillulae* has been fully described by Karling (96).

Six other species of *Harposporium*, all attacking nematodes, have been described from England (26, 27) and America (48, 60, 69). In *H. oxycoracum* (48) the fertile hyphae are longer than in *H. anguillulae*, and so are the spores, and each spore has at its distal end a small droplet of apparently adhesive fluid; this latter feature also appears in *H. helicoides* (26, 48), with even longer spores. In *H. diceraeum* (48) the very small conidia are sharply twisted, while in *H. bysmatosporum* they are shaped like a human upper arm bone; the latter species also differs from the others in that infection of the nematode is by the mouth (60). The delicate *H. lilliputanum* (26) has rather long fertile hyphae; the conidia resemble those of *H. anguillulae* in shape and are very small. *H. subuliforme* has awl-shaped spores, from the tips of which short adhesive spurs are put forth obliquely (69).

The endozoic species of *Acrostalagmus* attack nematodes in the same manner as *Harposporium*. *A. obovatus* (48) is extremely

common; I have observed it in garden compost, soil, leaf-mould, decaying plant stems, cow-dung and rotting wood, and it is by no means uncommon in decaying plant material in water (Peach, personal communication). The mycelium consists of branched, septate hyphae which fill the integument of the nematode, having absorbed its contents. From the internal mycelium, fertile hyphae are given off as lateral branches which grow out from the carcass of the eelworm to a length of up to half a millimetre; they are irregularly septate and may be prostrate or somewhat ascending. The fertile hyphae bear at intervals flask-shaped phialides, formed singly or in groups, at the apex of which the slimy phialospores are produced successively, cohering round the distal end of the neck of the phialide in groups of up to twenty. The spores are ovoid to sub-spherical, and very small. The spores adhere to the exterior of passing nematodes, their germ tubes penetrating the integument and giving rise to the internal mycelium. As the spores are produced in large numbers, chances of infection are good, and in plate cultures nematodes are frequently seen moving about actively with many spores adhering to their skins, especially near their anterior ends.

Several other species of *Acrostalagmus* are known to attack eelworms. In *A. gonioides* (48) the fertile hyphae stand erect or ascending, and the phialides on the aerial portions are usually arranged in whorls. *A. zeosporus* (58) has phialospores shaped somewhat like maize grains, while in *A. bactrosporus* (48) they are cylindrical. In both *A. tagenophorus,* which attacks rotifers, and *A. gonioides* in eelworms, chlamydospores may be present (50), formed on short hyphae outside the host.

The validity of the genus *Acrostalagmus* is open to doubt. Those species in which the phialides are arranged in whorls could be accommodated in *Verticillium,* but *Acrostalagmus obovatus,* where the phialides are not verticillate, is difficult. Since all these species are clearly related and have pronounced characters in common, the best solution would probably be to place them in a genus on their own.

Verticillium sphaerosporum, recently described as attacking the stem eelworm (*Ditylenchus dipsaci*) in diseased *Calceolaria* plants from Scotland (86), closely resembles *Acrostalagmus obovatus,* but its spores are more nearly spherical and the phialides are

mainly in whorls on ascending fertile hyphae. Another not uncommon fungus, closely related to the various species of *Acrostalagmus,* is *Spicaria coccospora* (48); it differs from *Acrostalagmus* in that the conidia, instead of forming a group round the tip of the phialide, are in chains. Like *Acrostalagmus,* the validity of the genus *Spicaria* is dubious.

Cephalosporium balanoides (48) resembles *Acrostalagmus obovatus* in its general organisation, and also attacks nematodes. It differs in its phialides, which are cylindrical rather than flask-shaped, on long prostrate fertile hyphae. The spores are markedly triangular in outline.

The species of *Acrostalagmus, Verticillium* and *Spicaria* described above, and, perhaps, of *Cephalosporium balanoides* appear to form a closely related group; they should probably be regarded as congeneric. Like them in vegetative organisation but totally different in its manner of spore production, is the interesting fungus *Meria coniospora* which has been recorded attacking nematodes both in England (73) and America (48). Infection of the host is brought about by adhesive spores, and the usual endozoic mycelium of septate hyphae develops. After death of the animal, rather short fertile hyphae emerge; these are procumbent or ascending and bear small conical spores on fine sterigmata. The spores are flattened at their broad basal end and pointed at the apex which often bears a tiny globule, possibly of some sticky substance to enable the spore to adhere to the skin of a nematode. Nematodes can often be seen in cultures, moving about with large numbers of the conidia sticking to them, and the conidia are not removed by the worms brushing against solid objects that lie in their path. The production of spores on sterigmata instead of on phialides seems to place *Meria coniospora* rather apart from the endozoic hyphomycetes so far described.

Far removed from the other predacious hyphomycetes is the genus *Nematoctonus* with its relatively large conidia formed on lateral sterigmata and clamp connections on its fertile hyphae. *N. tylosporus* (48) forms a mycelium of branched septate hyphae in nematodes, following infection by an adhesive spore. After death of the host, fertile hyphae grow out from its carcass to a distance of a millimetre or more. They bear fusiform conidia on short lateral sterigmata; these conidia, which are of the order of

20 microns long and two or three microns wide, are more massive than those of any of the endozoic species described above. The conidia are attached to the sterigmata by their broader proximal ends, while at their distal ends they carry a small knob which is probably adhesive. Sometimes the knob is replaced by a short process, probably formed by premature germination, which according to Drechsler (48) is highly adhesive. By means of either the knobs or, more usually, the apical processes, the conidia stick to nematodes and, on germination, the skin of the animal is penetrated; from the penetration tube the internal mycelium is derived. In addition to the conidia, the fertile hyphae may bear ovoid, yellowish, spore-like bodies. These are probably resting spores.

The most interesting feature of *N. tylosporus* is the presence of well-formed clamp connections on the fertile hyphae. These differ in no way from the typical clamp connections of the Basidiomycetes, and suggest strongly that *Nematoctonus* may be an imperfect representative of that group. Nothing is recorded concerning the nuclear condition in *Nematoctonus;* in view of the presence of clamp connections, it would be interesting to know whether the cells are dicaryon. Several other species of *Nematoctonus* have been described (48, 52, 56, 66), all attacking nematodes.

OTHER PREDACIOUS FUNGI. Nearly all the predacious fungi that are at present known are referable to one or other of the two major taxonomic groups which have already been described. There are, however, a few interesting phycomycetous species which, in their organisation for the predacious life, have many features in common with the Zoopagaceae and the predacious hyphomycetes, though they are unrelated to either.

Zoophagus insidians was one of the earliest predacious fungi to be described (1, 125). It has since been recorded from Austria (85, 125), Jugoslavia (85), France (111), Bulgaria (1, 128), America (95, 126) and England (4). The mycelium consists of fairly delicate, non-septate hyphae, occurring in water, often among and upon algae. The hyphae bear at intervals short, unbranched, peg-like processes, by means of which rotifers are captured. The tip of the process bears a mucilaginous secretion to which rotifers appear to stick, a hyphal outgrowth from the fungus then giving

rise to a tangle of absorptive hyphae within the animal. In this mechanism *Zoophagus* shows analogy both with the Zoopagaceae and with hyphomycetes such as *Dactylella lobata* and *D. cionopaga.*

Accounts of reproduction in *Z. insidians* are conflicting in the extreme. Arnaudow (1) describes the formation of biflagellate zoospores matured in a vesicle extruded from a filamentous sporangium, while Giklhorn (85), who also observed biflagellate zoospores, claims that the sporangium was globular and that no vesicle was formed. Still more confusing is the later description (3) of bodies which were probably conidia. This observation is particularly interesting, since it may well be that two or more different fungi have been concerned and that one was a member of the Zoopagaceae. Some uncertainty is also attached to the sexual reproduction in *Z. insidians;* fertilisation of a spherical oogonium by an antheridium has been described, but here again the possibility of confusion can not be ruled out of account.

Zoophagus tentaculum has been described from America (95). Here the organs of capture consist of stout lateral outgrowths, each having at its apex a number of slender tentacles. This species reproduces by means of fusiform conidia, formed acropetally on long hyphae, suggesting strongly that its affinities lie very close to the Zoopagaceae. Sparrow (127) places *Zoophagus* tentatively in the Pythiaceae.

Another rotifer-capturing phycomycete, *Sommerstorffia spinosa,* was first observed near Sofia (2). Rotifers were infected by spores which entered by the oral passage; these germinated and produced an endozoic mycelium from which hyphae grew out of the dead host into the surrounding water. These hyphae bore lateral branches, the tips of which were engulfed by other rotifers, which became captive. Spores were produced by the mycelium within the captured animal and discharged to the exterior. The spores encysted at the mouth of the discharge tube, later producing biflagellate swarmers. Sexual reproduction, by fertilization of an oogonium containing a single oosphere, was also recorded.

A quite different kind of organisation is found in *Protascus subuliformis* (11, 73, 97, 109, 110) which is endoparasitic in nematodes. Infection is achieved by one of the club-shaped non-motile spores of the fungus becoming attached by its pointed end to the integument of a nematode. The contents of the spore pass into the animal, forming within it a subspherical or ovoid mass of

protoplasm which gradually grows and elongates to form a wide, irregularly filamentous thallus, which later becomes divided by a few septa. The thallus grows at the expense of the animal which at first seems little the worse for the presence of the parasite. Eventually, however, the nematode becomes increasingly sluggish and stiff in its movements and finally dies, usually in an extended position. In the later stages of its development the thallus of the fungus breaks up into its individual cells, each of which may become a sporangium or a gametangium.

During the vegetative phase the segments of the thallus are filled with cytoplasm, but as the time for development of spores approaches, a large central vacuole appears, the peripheral cytoplasm then segmenting to form spores. These are club-shaped, slightly curved, with the wider end bluntly rounded and the narrower end pointed, and they are usually arranged in the sporangium with their rounded ends towards the exit canal. This develops while cleavage in the sporangium is beginning; a process from the sporangium grows out through the integument of the dead animal to the exterior and, when the spores are ready for discharge, its tip opens, apparently by deliquescence. The spores are ejected forcibly, and usually when the nematode has died on the surface of an agar culture the ejected spores lie in a compact group, well clear of the mouth of the exit tube. The mechanism of this ejection is not clear; it has been suggested (110) that the spores are forced out by cytoplasmic contractions in the sporangium, but in the specimens that I have observed it did not appear to me that there was sufficient residual cytoplasm after delimitation of the spores to accomplish this. Possibly some turgor mechanism is involved, a suitable osmotic force being provided perhaps by hydrolysis of some substance such as glycogen.

Spores are produced in large numbers and, lying on the surface of the substratum, they are readily picked up by nematodes. They adhere firmly to the integument of the eelworm by their pointed ends, probably by virtue of a sticky secretion, though no adhesive fluid is visible under the microscope. In agar cultures infected by *Protascus* it is a common sight to see nematodes moving freely with 20 to 30 spores attached to them, mostly at the anterior end. In older cultures, where there are plenty of spores about, multiple infection is common.

Sexual reproduction takes place by conjugation between two

thallus segments; whether the conjugating segments belong to the same or to different thalli is difficult to observe. Protuberances put out by the two conjugating segments meet and fuse, the contents of one gametangium passing into the other. The sexual process is usually slightly anisogamous. The oospore, rounded and with a thick wall, develops within the "female" gametangium which is usually slightly the larger of the pair. The zygote then passes into a resting stage and is finally set free by decomposition of the remains of the nematode. In old cultures lines of resting spores often mark the spot where a nematode has been killed, all traces of the animal itself having vanished.

Protascus subuliformis is probably best placed in the Lagenidiales (97) but is separated from most other members of the group by its non-motile spores. Transition from a zoospore to an aplanospore is a logical step for a fungus parasitic in a host which is both highly gregarious and actively motile; in such circumstances spore motility might even be a biological disadvantage compared with the greater efficiency of an adhesive spore, and it seems worthwhile to note here the curious fact that, although eelworms are common in moist habitats, very few of the numerous fungi known to attack them have motile pores.

Myzocytium vermicolum, also parasitic in nematodes, has many features in common with *Protascus* and was first described 70 years ago (130). As in *Protascus,* the thallus within the nematode consists of a stout, irregular filament, which becomes divided at maturity into a number of cells, the filament being somewhat constricted at the septa so that the individual cells are barrel-shaped in outline. The cells do not separate. Each cell becomes a zoosporangium, the cytoplasm surrounding the central vacuole cleaving to form biflagellate zoospores, while a lateral process from the zoosporangium develops into an exit canal, growing through the integument of the dead host and liberating the zoospores outside. The zoospores, in the specimens that I had under observation, completed their development in the zoosporangium, no vesicle being formed; this conflicts with the account given by the author of the species (130) but agrees with a later report from France (12). After liberation the zoospores swarm for a few minutes, after which they usually encyst. Not uncommonly, instead of swarm spores emerging from the zoosporangium, non-motile bodies are formed, having the appearance of zoospores that have ger-

minated to produce a yeast-like bud at one end. Often two or three of these bud-like structures are formed in a chain. Whether these are in fact zoospores that have germinated (97) or whether they should be regarded as aplanospores remains to be determined; the latter view accords with the fact that in the two probably closely related species, *Protascus subuliformis* and *Haptoglossa heterospora,* the spores are always non-motile. The development of an aplanospore from a zoospore might well be an adaptation to parasitism of such a gregarious and highly motile host as a nematode. Whatever the nature of these bud-like excrescences, they appear to act as adhesive organs for attachment of the spore to the host, for I have observed spores clinging to nematodes by means of them.

Haptoglossa heterospora was first described from America by Drechsler (46), and has recently been observed in Denmark by Shepherd (personal communication). I have found it in soil in England. It forms an ovoid or rectangular thallus in nematodes, a single eelworm often containing numerous thalli. It is holocarpic, the whole thallus becoming a sporangium at maturity. The spores are globular or angular; they are non-motile, and each spore is provided with a lobed "infective body" by means of which it adheres to the integument of a nematode, thus recalling the bud-like structures in *Myzocytium vermicolum.* A remarkable feature of this fungus is that it has spores of two sizes; a given sporangium may contain large or small spores, but apparently not both. The reason for this peculiar habit is not known.

A remarkable member of this series of endozoic Phycomycetes is *Gonimochaete horridula* (59). The thalli are parasitic in nematodes, often large numbers of them in a single worm. The exit tubes are long and hypha-like, often ascending from the carcass of the host and presenting a bristly appearance. The spores are cylindrical and small, and are usually expelled, not very forcibly, from the exit tubes, two, three or four at a time; they may be held aloft for a while at the end of the exit tube. After discharge each spore develops a bulbous outgrowth which is adhesive, enabling the spore to stick to the exterior of the host.

Protascus subuliformis, Myzocytium vermicolum and *Haptoglossa heterospora* are probably best placed together in the Lagenidiales, though it is not here suggested that they are closely

related. They differ from other members of the alliance in their non-motile spores, but it has been suggested that this modification may well be an adaptation to their requirements for successfully attacking nematodes. The long exit tubes of *Gonimochaete* are remarkable, and it is difficult to see what biological advantage, if any, they secure for their possessor. *Gonimochaete* is placed by its author tentatively in the Entomophthorales, but in its holocarpic organisation and endogenous spore production it shows strong affinities with the Lagenidiales.

Three Lagenidiales which attack rotifers have been described from Brazil (98). These are *Lagenidium microsporum, L. parthenosporum* and *L. distylae,* all occurring in the Amazon valley. All three of them parasitise species of *Dystyla,* and *L. parthenosporum* also occurs in nematodes.

Lastly in this phycomycetous endozoic series we have *Meristacrum asterospermum.* The mycelium within living nematodes consists of a broad filament which may be as much as half a millimetre long, but which normally breaks up into segments with rounded ends. Each segment may form conidia or an azygospore. The conidiophores produced from the segments emerge from the host and rise erect into the air, bearing laterally sessile obovoid conidia which are formed in basipetal succession. These conidia, when abstricted, adhere to nematodes and thus spread the infection. Sometimes, instead of a fertile hypha bearing conidia growing out from a segment, the protoplasm of the segment collects into the centre of the cell and, rounding off, is surrounded by a somewhat undulating wall enclosing a subspherical protoplast. This is evidently a resting spore and is probably to be regarded as an azygospore.

The segments into which the mycelium of *Meristacrum* divides appear to be comparable with the hyphal bodies characteristic of many of the Entomophthorales, such as *Empusa muscae.* Affinity of this species with the Entomophthorales is further supported by the asexual reproduction by means of conidia, and by the presence of azygospores.

PHYSIOLOGY

The physiology of the predacious fungi has been very little studied, most of the work that has been done having been concentrated on the trapping mechanisms of the predacious hyphomycetes. Such problems as the special nutritional requirements

that may have resulted in or resulted from the predacious habit have not been touched. An interesting field for research is vacant here.

The networks, adhesive knobs, constricting rings and other adaptations by which the predacious hyphomycetes capture their prey have long been a source of interest, though the volume of work that has been done on their physiology is relatively small. It is well known that most of these fungi do not form traps when grown in pure culture, trap formation being initiated when nematodes are added. Some species, however, appear to be able to produce traps independently of the presence of eelworms, although more traps may be formed if worms are present. *Dactylella cionopaga* produces its adhesive branches in pure culture on maize-meal agar, but the rather similar *D. lobata* does not (79). *D. ellipsospora* is another species that does not always depend on the presence of nematodes for the formation of its adhesive knobs. Reticulate species normally do not form networks in pure culture, but I have recently found that a number of isolates of *Arthrobotrys robusta* and *Trichothecium cystosporium* in my own culture collection are showing a strong tendency to do so, though the reason for this unusual behaviour is not apparent. Species with constricting rings do not normally form their traps without appropriate stimulation, though an occasional ring may be seen in pure culture.

The presence of actual nematodes is not necessary for trap formation, for it has been found that a sterile filtrate of water in which nematodes have lived will usually act as a stimulus. This was very clearly demonstrated by Comandon and de Fonbrune (8). These workers experimented on four nematode-trapping hyphomycetes, *Arthrobotrys oligospora, Dactylella bembicodes, D. ellipsospora* and *Dactylaria brochopaga,* which they grew in pure culture on beer-wort agar. Spores were formed abundantly but not organs of capture, except sometimes by *D. ellipsospora.* Traps were numerous in eelworm-infected cultures.

The part played by nematodes in the initiation of the traps was verified by adding living sterile nematodes to the cultures, the sterile worms being obtained by isolating eggs from soil and sterilizing them by treatment with 10% hypochlorite solution. After some hours the eggs hatched, and the young worms could live for several days on agar without contamination with bacteria.

It was found that addition to cultures of the fungi of a little water in which sterile nematodes had lived was sufficient to stimulate trap formation, but that both nematodes and water lost this activating power after boiling. It would seem, therefore, that the stimulus to trap formation was due to some diffusible substance produced by the eelworms, and that this substance was heat-labile.

Later experiments in America (10), using *Dactylella bembicodes* as the test fungus, have suggested that the pH of the medium may play a part in constricting ring formation. The fungus was isolated from semi-decayed pine wood, where it formed abundant mycelium and many rings, into pure culture on maltose peptone agar. No rings were formed in pure culture. Addition of a drop of the brownish water from the original dish containing nematodes and fungus caused ring formation to occur within 24 hours. It was observed that this water was acid, so the effect of acidifying pure cultures of the fungus was tried. Addition of 0.1 ml., 0.2 ml. and 0.5 ml. of deci-normal phosphoric acid, respectively, to 20 ml. of maltose-peptone agar had no effect; but when 1 ml. of acid was added, many rings appeared. Other experiments on the same lines showed that growth was more abundant and trap production greater on Bladeslee's No. 230 agar than on acidulated maltose-peptone agar, while on potato dextrose agar growth was still more luxuriant but ring formation was suppressed. It was also observed that trap formation increased as available food in the medium was reduced.

Further experiments in France have extended the list of substances capable of stimulating trap formation (120). Working on *Dactylella bembicodes,* the French experimenters attempted a quantitative evaluation of the effect of various substances in stimulating ring formation, the potency of a given substance being estimated by the number of rings produced on a ten-centimetre Petri dish. Having observed that infection of the cultures by certain bacteria could act as a stimulus, they used sterile solutions in some of their experiments. They found that sterile human blood serum was a powerful stimulus to trap production, while sterile rabbit serum and sterile human urine were less effective. In a later series of experiments (22) a number of vegetable extracts was tried, always with negative results, while various substances of animal origin, namely, serum, extracts of different organs and even certain living unicellular organisms such as

Euglena gracilis, reacted positively. Guinea pig serum gave a positive reaction in a dilution as high as one part in a thousand when tested against *D. bembicodes,* and the active principle was found to be thermostable, resisting a temperature of 120° C. for one hour and a half; it was not entirely destroyed by treatment with alcohol.

Drechsler (30) has observed that constricting ring formation in pure cultures of a predacious hyphomycete, probably *D. bembicodes,* was initiated as a result of infection of the culture by mites, and he suggested that the effective factor was friction on the mycelium, produced by the mites walking across it; in view of the French work, however, it is possible that the presence of animal protein or some other substance brought in by the mites was the causative agent. The same phenomenon has been observed in mite-infested cultures of *Dactylella acrochoeta* (71).

The operation of the traps in the predacious hyphomycetes was the subject of brilliant research by Comandon and de Fonbrune (8), using a very sensitive pneumatic micromanipulator of their own design. Four hyphomycetes were used in these experiments —*Dactylaria brochopaga, Dactylella bembicodes, D. ellipsospora* and *Arthrobotrys oligospora.* They also used *Stylopage hadra,* a nematode-capturing member of the Zoopagaceae which does not form specialised traps. Microcultures of the fungi were prepared on a thin cellophane membrane soaked in nutritive fluid and spread out beneath the cover glass in an oil chamber; when the mycelium was sufficiently developed, nematodes were introduced, and traps were formed in sufficient quantity in about 24 hours.

Experiments on the constricting rings were conducted mainly with *Dactylaria brochopaga* as test fungus, but the mode of action appeared to be the same in *Dactylella bembicodes.* To observe the capture of an eelworm under the microscope, one of the worms was picked up in a micro-pipette made so that its cavity had a diameter less than that of the middle of the worm, which was retained by suction with its posterior end in the pipette. The nematode was then introduced into the ring by means of the micromanipulator. On rubbing the inside of the ring with the eelworm, the ring closed quickly by the swelling of the three cells of which it was composed, and gripped the nematode so firmly that the mycelium could be torn without disengaging the worm.

It was found possible by mechanical stimulation to make the

traps work without the presence of nematodes, and this enabled movements inside the cells of the ring to be studied under the highest powers of the microscope. A ring was placed by the micromanipulator horizontally in contact with the lower side of the cover glass of the oil chamber in order to facilitate microscopical observation. If the outside of the ring was rubbed with a micro-needle, nothing happened, but if the needle was inserted inside the ring and one of the three cells was gently rubbed, the stimulated cell quickly swelled towards the inside. Cinematographic measurements showed that its volume was more than trebled in less than one tenth of a second. Usually, a fraction of a second later, the other cells of the ring reacted without having been touched, indicating that the tactile stimulus could be transferred from cell to cell. On closure of the ring the needle was gripped so tightly that it could not be withdrawn.

Study of cinematograph films taken during the experiments showed that the increase in volume was accompanied by rapid swelling of a number of vacuoles in the cells of the ring, which took place some seconds after the explosive dilatation of the trap. A captured eelworm removed complete with ring and placed on sterile agar was absorbed, and a new mycelium was formed from hyphae derived from the ring.

The sticky mechanisms of *Dactylella ellipsospora, Arthrobotrys oligospora* and *Stylopage hadra* were studied by similar methods. It was observed that *D. ellipsospora* sometimes formed its adhesive knobs in pure culture, but that they were more numerous in the presence of eelworms. Nematodes placed by the micromanipulator in contact with the knobs adhered rapidly and firmly, and in a few seconds could not be detached. Not all the knobs were equally sticky, and it appeared that adhesiveness reached a maximum at a certain period in the life of the hyphae that bore them. Certain species of nematode did not stick to knobs that were highly adhesive for others. Only nematodes, among all the objects tested, stuck to the knobs; these objects included a glass microneedle, the chitinous exoskeleton of insects, the surface of tiny oligochaete worms and various other things.

With *Arthrobotrys oligospora* it was found that the sticky networks were formed only in the presence of nematodes. The whole surface of the network was adhesive. The observations made with

D. ellipsospora were true in general of *A. oligospora;* all species of eelworm were not caught, there was an optimum age for adhesiveness, and objects other than nematodes did not stick. When, however, the networks were removed from a moist medium—i.e., left exposed to the air—it was found that they were adhesive to a glass needle and that a sticky substance which hardened rapidly was visible.

When a point on the cell-wall of one of the cells of a network was gently rubbed with the point of a micro-needle, the protoplasmic granules near that point were observed to move faster, and this excitation was communicated to the entire protoplasm of the cell and sometimes to that of adjacent cells. At the same time these granules moved towards the point stimulated and collected round the edges of large hyaline vacuoles that were present in the cells. A similar phenomenon was observed in *Stylopage hadra.*

S. hadra possesses no specialised organs of capture, but the whole surface of the mycelium was found to be adhesive for certain species of nematodes. Adhesiveness reached a maximum at a certain stage in the growth of a mycelial filament. Eelworms coming into contact with a hypha of the fungus appeared to be held by the secretion, around the point of contact, of a substance which solidified rapidly; the existence of such a substance has been noted by Drechsler (34). Local stimulation of the surface of a hypha produced a protoplasmic reaction similar to that observed in *Arthrobotrys oligospora;* but here, the hyphae being non-septate, the excitation was transmitted to some distance on each side of the point stimulated, with a measurable velocity. The reaction could be produced by rubbing with, or pressure by, a micro-needle, and it seems as though the accumulation of granular protoplasm that results is related to the secretion of the sticky substance that holds the nematode.

A year before the work described above, some less extensive experiments were carried out in America (10) on the mode of operation of the constricting rings in *Dactylella bembicodes.* Nematodes were added to a Petri dish culture of the fungus, and their capture by the constricting rings was observed microscopically. In fresh cultures, closure of the rings was very rapid, but in old cultures they appeared to lose their sensitivity. Since

the vigorous movement of nematodes suggested that the stimulus producing closure of the rings was a mechanical one, this was tested by inserting a fine glass hair into a ring under the microscope, the hair then being moved backwards and forwards to imitate the movement of a nematode. In no case could more than a slight swelling of the ring cells be obtained. This observation is in direct contradiction to the findings of the French workers, and it is hard to understand why this should be so. One can only say that nobody who has seen the remarkable film produced by Comandon and de Fonbrune could reasonably doubt the accuracy of their statement that closure of the rings follows stroking with micromanipulator needle. Probably some slight difference in the physiological state of the cultures used by the experimentors would account for the discrepancy.

Observations were also made on the effect of certain chemical substances on the rings. Weak solutions of potassium hydroxide and also of phosphoric, acetic, hydrochloric, sulphuric and lactic acids were applied to the rings. It was found that treatment with 1% lactic acid produced a slight swelling of the ring cells, but in no instance could complete closure be obtained. It was, however, found that complete closure of the rings could be produced by heat, applied by dropping water at a temperature between 33° C. and 75° C. on to them; below 30° C. no swelling occurred, while about 80° C. the cells were killed. Dry heat from a hot needle held near the rings was also effective. Cells swollen by hot water or dry heat were seen to contain a refractive substance, thought to be gelatinous or colloidal in nature, and it was suggested that swelling of the cell may be due either to rearrangement of molecules of water and colloidal material already in the cell, or to imbibition of additional water from the stalk; the speed at which closure took place was considered to favour the first view.

While the experiments described above shed a great deal of light on the nature of the stimuli that can induce closure of the rings, the mechanism by which the swelling is actually achieved is still a complete mystery. The speed of the reaction is against the idea of a turgor mechanism dependent on intake of water either from the hypha subtending the ring or from the surrounding medium, and the theory that the phenomenon results from molecular rearrangement of cell colloids, probably involving water

already contained in the cell, seems to be the more likely. Such mechanisms are known, or suspected, to exist in the higher plants, and could at least theoretically occur in the fungi. The constricting ring mechanism offers an attractive line of research for a plant physiologist, with the possibility that the knowledge of cytophysiological phenomena gained in elucidating the problem might have a far wider sphere of application than that of the predacious fungi alone. In this connection it might not be unduly fanciful to suggest that the mechanism of the constricting ring could be of more than passing interest to the student of stomatal movement in higher plants.

Apart from *Stylopage hadra,* the means by which the mycelial Zoopagaceae capture Protozoa have not been experimentally studied. Secretion by the hyphae of a yellowish adhesive material has been reported on a number of occasions by Drechsler, and it enters into his diagnoses of some of the genera (33). This adhesive substance is not always visible. Amoebae can cling with great tenacity to solids; it has been stated (87) that they may even remove minute fragments from a glass surface on which they are moving. Amoebae in plate cultures have an irritating habit of ranging themselves alongside fungal hyphae, simulating capture by predacious fungi where none exists, and thereby wasting the time of the observer; it is not impossible, therefore, that in some instances it may be the amoeba that is clinging to the fungus instead of the converse. This certainly seems to be the way in which capture of *Geococcus vulgaris* by *Dactylella passalopaga* (38) is effected. I have observed that old mycelia of *Stylopage rhicnacra* (77) and other Zoopagaceae often have the remains of captured amoebae, whose contents have been almost completely absorbed, attached only by the haustoria, all contact of the ectoplasm of the amoeba and the main hypha of the fungus having been broken. While one could not justifiably infer from this the absence of sticky material, it is suggestive.

The temperature relations of predacious fungi have not been studied, though some incidental observations by Drechsler are interesting. He points out that cool humid weather is favourable for the development of predacious fungi in laboratory cultures (64), a statement with which I wholly concur, although individual species vary in their preferences. *Dactylella gephyropaga* is re-

ported to grow actively and to capture nematodes abundantly at temperatures of 25–30° C., while *D. cionopaga* is almost totally inactive (67); at 15–18° C. the effect is reversed. This may be correlated with the northern distribution of *D. cionopaga* compared with *D. gephyropaga* (67). In the same way *Dactylaria psychrophila* appears to be more heat-tolerant than *D. thaumasia*. The development of thalli of *Cochlonema agamum* in amoebae is said (57) to occur rapidly at 23° C., while reduction of the temperature to 15° C. seems to inhibit the fungus and to favour recovery of the amoeba.

The constricting rings of the predacious hyphomycetes appear to have a strong tendency to take up a position perpendicular to the substratum. The biological advantage of this is clear, since it places the ring in the best position for ensnarement of nematodes, but the mechanism that ensures correct orientation has not been investigated. Geotropism can hardly be involved as the surface may not be horizontal, and the existence of a phototropic response, besides being unlikely, would seem to be ruled out, since the orientation of the rings does not depend on illumination of the cultures. Thigmotropism suggests a possible answer, and, since the rings are often placed just below the surface, a response to an oxygen or carbon dioxide gradient is not impossible. An investigation of this phenomenon might produce interesting results.

Apart from the erect position of the fertile hyphae in most species, tropic responses in the predacious fungi do not seem to be important, but it has been suggested (57) that development of the conidial chains of *Cochlonema agamum* may be positively phototropic.

The nutrition of the predacious fungi is a subject about which more information is badly needed. The endozoic species, both in the Zoopagaceae and in the hyphomycetous series, appear to be obligate parasites and have never been isolated in pure culture. Among those that capture living prey, the mycelial Zoopagaceae behave as strict predators, as Drechsler (62) points out. I have myself attempted to isolate *Stylopage rhynchospora*, *S. hadra* and a hitherto undescribed species of *Stylopage* that captures nematodes, taking advantage of the long erect conidiophores of these species to pick off spores under the microscope on a sterile needle and to transfer them to plates of maize-meal and of rabbit-dung agar. In

no instance was I successful, although in the original cultures fallen conidia were germinating freely. It is possible that germination of the spores depends on the presence of a chemical substance emanating for the host, analogous with the stimulus needed for trap formation by the predacious hyphomycetes. Such a mechanism might be of advantage to the fungus by ensuring that conidia germinate only in the presence of suitable prey, an important point for an obligate predator.

In contrast to the Zoopagaceae, the nematode-capturing hyphomycetes grow well in pure culture on most ordinary media. Both mycelial bulk and density of sporulation are usually greater than when they are growing in competition with the bacteria and other fungi that are incidental to the presence of their hosts. An animal diet is not necessary for their existence, and many predacious hyphomycetes, when growing strongly in pure culture on a rich medium, may become relatively indifferent to nematodes when the animals are offered to them. On the principle that a hungry cat is the best mouser, they should be maintained in culture on a weak medium; maize-meal agar, without added sugar, appears to suit most of them very well. In pure culture the conidia are often smaller than those formed in the presence of nematodes; thus the spores of *Arthrobotrys oligospora* have a size range of 22–32 × 12–20 μ when formed in nematode-infested cultures, while in pure culture on maize-meal agar this is reduced to 18–30 × 10–15 μ (40). The habit of sporulation may also be different in pure culture. The fertile hyphae of *Arthrobotrys oligospora* in pure culture bear their conidia in discrete whorls separated by "internodes", the number of whorls often being a dozen or more; but when the fungus is feeding on nematodes, the conidia tend to form a single terminal head, the additional whorls appearing only in older cultures, and then being few in number. The same phenomenon occurs in *A. superba* and *A. conoides*. The reason for this is uncertain. The high nitrogen to carbon ratio resulting from an animal diet does not seem to be the operative factor, for I have found that multiple whorls of conidia are still formed in pure cultures containing meat extract or peptone, while on some high carbohydrate media, such as potato dextrose agar, sporulation may be much reduced with concomitant excessive mycelial growth.

The pH requirements of the predacious fungi do not appear to

have been studied. Adjustment of the reaction of the medium does not appear to be necessary in culturing them, and this applies both to the Zoopagaceae and to the hyphomycetes. I have observed some indication that in Nature they are less frequent in acid peaty soils, even though suitable hosts may be plentiful, but I have not sufficient data to justify any generalisation about this. Some recent laboratory experiments (Dixon, unpublished) suggest that different species of predacious hyphomycetes may differ radically in their growth reaction to changing pH, but the work is of a preliminary nature only, and no positive conclusions can yet be drawn.

An interesting correlation exists between the growth rate of the predacious hyphomycetes and the type of trap produced. Species having adhesive networks are usually faster growers than those with stalked knobs, while the constricting ring formers are slowest of all. This can be seen both in pure culture and in the presence of nematodes; in eelworm-infested plates, *Arthrobotrys oligospora* will usually make its appearance some weeks before *Dactylaria gracilis*. There does not appear to be any obvious reason for this correlation, but it does not seem unreasonable to suppose that for the production of extensive systems of networks, a vigorous habit of growth would be an advantage.

ECOLOGY

The habitat relations of the predacious fungi have been very little studied. This may in part be attributed to the general opinion that the ecology of the fungi is coincident with that of the hosts. While this assumption may be true up to a point, the evidence available at present is not sufficient to justify its adoption without investigating further, and recent unpublished work on predacious fungi in soil suggests that it may be very far from the truth.

Predacious fungi are widespread in Nature. They have been found in leaf-mould, rotting wood, decaying vegetable remains of many kinds, soil, dung, decaying plant material in water, and on and among bryophytes (78). A fungus assigned (probably incorrectly) to *Oidium* has even been reported from *Monoposthia duodecimalata*, a free-living marine nematode (7), and an internal parasite of copepods, recently observed by Sleigh, may possibly

be the first marine record of the Zoopagaceae. They may occur in the most unlikely places; a collection of soil from what was once the cellar of a bombed building in the middle of London yielded *Arthrobotrys robusta* and *Trichothecium cystosporium* (80), and in a small clump of moss (*Bryum argenteum* L.) from between paving stones outside a house several species were found (76). A recent survey (78) has shown little correlation between different kinds of habitat and the species of predacious fungi that occur, but it must be emphasised that much work will need to be done before any such correlations, if they exist, can be postulated with safety.

Leaf-mould as a substratum for predacious fungi has been widely explored by Drechsler, who has described a large number of species from this source. As a habitat for predacious fungi, leaf-mould has certain obvious advantages. An abundant fauna of nematodes and Protozoa is present, the moisture content is reasonably high and complete drying out will occur only under extreme conditions; the pH in leaf-mould from deciduous woods, moreover, is usually not too low. A certain amount of shelter is provided during hot weather, the temperature on the floor of a wood being lower than that on the ground outside. It is not surprising, therefore, that the list of species from leaf-mould is a long one. I have myself found it a good source of predacious fungi in Britain, though not so good as some others such as moss or rotting wood.

Partly decayed plant remains often contain predacious fungi, especially when lying on or near the soil. Garden compost, pieces of stem and leaf that have lain under stones, etc., old cabbage stumps and other vegetable bric-à-brac in a fairly advanced stage of decomposition, have all yielded their quota of predators. I have been fortunate enough to be able to examine some of the vegetable composts used by the late Dr. M. C. Rayner and her colleagues (117) in their work on mycorrhizal fungi in conifers, and found them very rich in predacious species, including *Trichothecium flagrans* (74). It is of some interest that this species has been detected on only two other occasions; once from garden compost at Kingston-on-Thames and once from tomato haulm compost from Manor Nurseries, near Kenilworth.

Rotting wood is one of the most prolific sources of predacious

fungi that has yet been brought to my notice. Old tree stumps, especially from fairly moist places, rotting logs lying on the ground in woods and pieces of old bark have an extensive fauna, including numerous Protozoa and nematodes, and appear also to provide a congenial habitat for many species of predacious fungi. This source had scarcely been touched until recently (26), but will amply repay further study. No attempt has yet been made to find out what ecological factors are at work, but, apart from the liberal supply of prey, the presence of moisture and, perhaps, organic compounds from the wood merit consideration. It is of historical interest that *Dactylella ellipsospora* was isolated from rotting wood as early as 1886 (88).

The predacious fungus flora of dung has hitherto received less attention than it deserves, though a number of scattered records exist. *Monacrosporium leporinum,* probably synonymous with *Dactylella ellipsospora,* was found in hare dung in Bohemia at the beginning of the present century, but its predacious activity, if any, escaped notice (6). *Arthrobotrys oligospora* has long been known to be frequent in horse dung and is often present in the milky fluid that climbs up the sides of a glass vessel in which fresh moist horse dung is allowed to stand undisturbed. There is evidence that the species present change with age, though this successional aspect has not been worked out yet, and it has been my experience that old dung, provided that it has not dried up completely, is usually richer in species than new. Among interesting predacious fungi that I have encountered in old cow dung are *Dactylella heterospora, Cystopage lateralis* and *Protascus subuliformis,* while horse dung, intended for use as garden manure, yielded on two separate occasions, *Stylopage cymosa* and *Arthrobotrys conoides.* A recent communication (94) has listed further species of predacious fungi from dung, and suggests that there are more to come.

Examination of mosses for predacious fungi was first suggested to me by Dr. B. Barnes, and has proved most fruitful. That mosses possess a rich and varied fauna of minute invertebrates, including many nematodes and Protozoa, has been made clear by a recent and very detailed study in Denmark (112). These animals are preyed upon by a rich assortment of fungi, drawn from most of the important predacious series. The source of

most of the moss-inhabiting predacious fungi is probably the soil beneath, and it seems reasonable to suppose that they grow up the moss plants from below, assisted by or in response to the presence in the moss clumps of water or animal prey or both. According to the Danish account, the nematodes tend to follow the "water table" in the moss cushions, and it is probable that the fungi do the same, though this has not been tested. As an example of the richness of the moss flora, one small collection of mosses made some years ago from the banks of a lane in Devonshire contained no less than eight species of predacious fungi (78).

Since predacious fungi are so common on and among mosses, it might reasonably be suggested that at least some of them are parasitic on the bryophytes. Although I have examined numerous specimens where both Zoopagaceae and predacious hyphomycetes have been growing and sporulating vigorously on the leaves of moss, I have never found the slightest evidence for believing that the fungi were other than harmless epiphytes.

The occurrence of predacious fungi in soil is a subject that, until recently, has been badly neglected, and this is surprising in view of the possible importance of the nematode-capturing species as a natural restraining influence on plant parasitic eelworms. In an experimental plot in Hawaii the presence of no less than 15 nematode-attacking fungi has been noted (102), and that this is nothing out of the ordinary is shown by the results of the examination of the surface layer of soil from a five-rod plot near London, where 21 species of predacious fungi were found, of which 13 attacked nematodes (Dixon, unpublished). Recent work by Shepherd in Denmark, not yet in print, has shown that a large and varied predacious fungus flora exists in the soil of that country, and my own observations during the past year on arable soil in Britain have convinced me that predacious fungi are as numerous in the soil as they are elsewhere, if not more so. It is surprising that they have not been recorded more frequently by soil mycologists, but the methods of examination used are probably unsuitable for their detection.

The soil flora includes both Zoopagaceae and predacious hyphomycetes, the *Arthrobotrys* series and the endozoic forms being well represented. The number of species in a given soil sample does not appear to be less than one would expect to find in a

collection of leaf-mould, moss or any other material in which these fungi occur. The relative frequencies of the various species in soil appears in general to be much the same as elsewhere, as far as the limited information at present available can be trusted, but there are certain exceptions to this. In a series of 49 soil samples that I have recently examined, 82 records of nematode-attacking fungi were made. *Arthrobotrys oligospora* accounted for 21 of these records, and another 18 resulted from an unidentified member of the Zoopagaceae which captured nematodes by adhesion to the mycelium after the manner of *Stylopage leiohypha* and *Cystopage lateralis,* but which formed no spores. As I have never found this latter species in any other habitat, unless it is a sterile strain of a species already known, it may be a specialised soil inhabitant. The next most frequent species in the series was *Harposporium anguillulae,* with six records, followed by *Acrostalagmus obovatus* with five and *Arthrobotrys dactyloides* with four. This striking preponderance of two species over all others is unusual.

Use of dung as an agricultural fertilizer must necessarily have a profound effect on the predacious fungus content of the soil, at least for a certain period after dunging. This has not been properly investigated, but I have observed on one occasion that a dunged plot contained a different predacious flora from an untreated control, and that this effect was maintained for at least three years after application of the dung. If nematode-attacking fungi have any effect on the plant pathogenic nematodes in the soil, this factor is worthy of serious consideration by agriculturalists.

The occurrence of predacious fungi in water has been studied intensively by Peach (113–115), to whom my thanks are due for permission to quote unpublished work in this field. An examination of water, soil and vegetable debris from various kinds of aquatic habitat has shown that both the Zoopagaceae and the predacious hyphomycetes are by no means confined to land. Some species seem to be particularly common in water or wet places; thus *Acaulopage dichotoma* was found capturing amoebae in aquatic situations on no less than 19 occasions. It may be noted that this species was originally described from waterlily leaves (33). *A. tetraceros* appeared in 16 samples from 11 aquatic habitats; the specimens of this species seemed to be of a strain with

longer and narrower conidia than the type, probably best regarded as a distinct sub-species. *A. dichotoma* and especially *A. tetraceros* are species in which the distal end of the conidium is evacuated, in *A. tetraceros* the empty portion forming from three to six filiform appendages; it seems not unlikely that added buoyancy resulting from this evacuation may be of assistance in dispersing the spores in water. Among the Zoopagaceae recorded by Peach the genus *Acaulopage* was by far the most frequent.

The predacious hyphomycetes are also well represented in aquatic habitats. Nine species of nematode-trapping hyphomycetes were identified by Peach, all the principal types of trapping mechanism being represented. *Arthrobotrys oligospora* appeared to be less common than it is in terrestrial situations, and the most frequently recorded predacious hyphomycete in the area covered by the work was *Dactylella cionopaga.* This is interesting in view of the fact that Drechsler (40) has recorded that *D. gephyropaga,* a species that captures nematodes by means of columnar adhesive branches somewhat similar to those of *D. cionopaga,* occasionally includes rotifers in its diet. There is, however, no similar record for *D. cionopaga.*

A curious feature of the survey carried out by Peach is the complete absence of such forms as *Zoophagus insidians* from her records, in spite of the fact that her cultural methods included "baiting" with boiled cress seeds. It may be that the media used in subsequent treatment did not suit their development, but it must be borne in mind that if an inventory of all the known predacious fungi is taken, the lower fungi, with the exception of the Zoopagaceae, are numerically insignificant. This is in itself surprising and difficult to explain, though two suggestions might be made: in the first place, aquatic surroundings may not be favourable for the capture of animals by trapping mechanisms, and in the second, the relatively uniform conditions of life in water may well lack the essential stimulus to evolution needed for the development of the predacious habit in more than a very small number of aquatic fungi. Whatever the reason, however, the fact remains that the predacious fungi occurring in water appear to be mainly land forms that may in some instances (*Acaulopage* spp.) have secondarily adapted themselves to some extent for aquatic life. It must also be remembered, in view of the method of spore

formation in *Zoophagus tentaculum* (95), that *Zoophagus* may not be a primarily aquatic genus.

OTHER BIOLOGICAL CONSIDERATIONS

The predacious hyphomycetes in the main show little, if any, host specificity. Most of the *Arthrobotrys* series appear to be able to capture a wide range of eelworm species, and it seems likely that the most important factor in deciding whether a particular species of eelworm is suitable prey is size. Robust predators such as *Trichothecium flagrans* are well able to cope with nematodes more than a millimetre long, while some of the more delicate species like *Dactylella asthenopaga* seldom capture worms of more than a third of that length. The nature of the cuticle of the nematode may also be of importance, for it is possible that the sticky secretions of the fungi may vary in efficiency according to whether the surface of the eelworm is smooth or striated. If this is so, its effect would probably be most important during senescence of the mycelium, when the traps are beginning to lose their stickiness. Some of the predacious hyphomycetes may even capture animals other than nematodes; thus *Dactylella gephyropaga* has been recorded (40) as occasionally capturing the rhizopod *Trinema enchelys,* the animal being caught by the mouth by an adhesive process as a result of attempting to feed off the fungus. *D. gephyropaga* has also been known to invade the oospores of *Pythium butleri* by means of lateral branches from submerged hyphae.

The endozoic predacious hyphomycetes vary in their ability to parasitise different host species. Many of them are known to attack nematodes belonging to several genera, while others have been recorded as parasitising a single species only, though here it is possible that further records may enlarge their host range. Our knowledge of most of these forms is insufficient to allow generalisations to be made.

In sharp contrast to the polyphagy of the predacious hyphomycetes, most of the Zoopagaceae are highly host-specific. It is most unusual for more than one species of rhizopod to be captured by a member of this family (62). There are, however, recorded instances of this happening. *Acaulopage ischnospora* regularly captures two species of *Amoeba,* and the same two species are

victims of *Zoopage virgispora.* It has been suggested (63) that these two amoebae may have some similarity in the composition of their protoplasm of their pellicles. It is possible, though not quite certain, that *Acaulopage baculispora* may also attack two different amoebae (64), and in this connection it is interesting to note that *A. baculispora* and *A. ischnospora* are morphologically very similar, differing mainly in the length of their spores and the amount of distal evacuation.

The members of the Zoopagaceae that capture nematodes appear to be more catholic in their taste, size being probably the most important factor in determining whether a given species of nematode is suitable for prey. It is reasonable to suppose that the limitations governing the capture of nematodes by adhesive material operate similarly for such fungi as *Stylopage hadra* and for the predacious hyphomycetes.

An example of hyperparasitism affecting a predacious fungus is found in the observation (71) that *Dactylella helminthodes,* a non-predacious species which normally subsists on *Pythium* oospores, may sometimes attack the oospores of *Cochlonema megalosomum.*

The principal genera of the predacious hyphomycetes contain species that appear to have no predacious tendencies. Thus, in addition to *Dactylella helminthodes,* mentioned above, *Trichothecium polyctonum* also attacks *Pythium* oospores, while *Dactylella strobilodes* (68) appears to be purely a saprophyte. The well-known *Trichothecium roseum* also has never been known to attack animals. A number of other instances are known; it must, however, be remembered that in the past several fungi long known as saprophytes have ultimately been shown to be predacious. Different isolates of the same species, moreover, differ in their aggressiveness towards eelworms, so that it is always possible that one worker may succeed where another has failed in showing that a given species is predacious. I have known a particular strain of a predacious hyphomycete to lose all interest in nematodes, at least for a time, when cultured under the wrong conditions; for example, *Trichothecium flagrans* when grown in pure culture on maize-meal agar may lose its power of forming adhesive networks and with it, incidentally, its ability to produce conidia. Both these faculties may sometimes be restored by

culturing the fungus on rabbit-dung agar. I can offer no explanation of this curious fact.

Spore dispersal in the predacious fungi has not been investigated. Endobiotic species usually have adhesive spores, as in *Endocochlus, Cochlonema* and the endozoic hyphomycetes, with the exception of *Myzocytium vermicolum,* which has zoospores. It is worthy of note, however, that *M. vermicolum* also produces non-motile, adhesive spores, and it has already been pointed out that for attacking a highly motile and gregarious host such as a nematode, motile spores are unnecessary and probably less effective than an adhesive mechanism. Species that attack nematodes by means of adhesive spores, therefore, can afford to rely on the motility of their hosts to secure dispersal.

The conidia of the *Arthrobotrys* series and of the mycelial Zoopagaceae appear to be quite suitable for dispersal by air currents, but in view of their habitat, earthworms and arthropods should not be ruled out as possible agents. In mite-infested cultures I have often seen the conidia of *Arthrobotrys oligospora* and other predacious hyphomycetes attached in large numbers to the hairs on the bodies of the animals, especially at the posterior end. There is no evidence that these fungi ever attack mites.

In the genus *Acaulopage* the conidia are usually provided with empty distal appendages, formed by shrinkage of the cytoplasm followed by cutting-off of the appendage by a septum. Such appendages might be of assistance in air dispersal, or they might enable the conidia to stick to animals for transport. A third possibility here, however, and one that must be given very serious consideration, is water dispersal, for the work of Peach has clearly shown that some species of *Acaulopage* have distinct aquatic tendencies. The whole subject of dispersal in the predacious fungi needs investigation.

A study of the distribution of predacious fungi shows that different species vary greatly in their range. *Arthrobotrys oligospora* and *Dactylella ellipsospora* appear to be widely distributed in both hemispheres, while some species, as far as available records show, are much more restricted. *Arthrobotrys robusta* occurs in Britain in Middlesex, Surrey, Essex, Westmorland, Warwickshire, Lincolnshire and Cambridgeshire, and is evidently a very common fungus in this country; it has not, however, been recorded by

Drechsler, which suggests that it can not be widespread in the United States. *A. musiformis* seems to be more local in its British distribution; I have found it in leaf-mould from three stations in Warwickshire, all close together, and nowhere else. It has been recorded from Denmark by Shepherd. Among the Zoopagaceae, *Acaulopage tetraceros, Stylopage rhynchospora* and *S. rhicnacra* are common in Britain, while no species of *Endocochlus* or of *Bdellospora* has yet been recorded as British.

The origin of the predacious habit presents a problem of great difficulty. Leaving aside such internally parasitic forms as *Protascus, Myzocytium* and the endozoic hyphomycetes, we have in the Zoopagaceae and the *Arthrobotrys* series two groups of fungi, taxonomically quite unrelated, which have both adopted a highly unusual mode of life, and which show certain resemblances to one another in their adaptations; for instance, the production of a sticky secretion for the ensnarement of nematodes in *Stylopage* and *Arthrobotrys*. Any adaptive resemblances between the two groups must be homoplastic, but it is reasonable to speculate on whether the same or similar circumstances led to both groups adopting the predacious manner of life. If wide distribution may be taken as indicating antiquity, the original adaptation must have occurred a long time ago, for *Arthrobotrys oligospora, A. musiformis, Dactylella ellipsospora* and others have been recorded from Europe, America and Australasia. The analogy between the predacious fungi and the insectivorous flowering plants is a striking one (108), and it is by no means impossible that both these groups may have adopted their peculiar mode of life in response to similar circumstances. Difficulty in obtaining adequate supplies of some essential food substance suggests itself readily as a predisposing factor, and deficiency of nitrogen seems as likely as any, though the importance of a source of carbon can not be overlooked.

ECONOMIC ASPECTS

The importance of nematodes as destructive parasites of crop plants has led a number of workers to attempt the biological control of these pests by means of the predacious fungi. For these experiments, the hyphomycetes of the *Arthrobotrys* series have been mainly used, as neither the endozoic hyphomycetes nor the Zoopagaceae lend themselves to artificial culture on the scale

needed for the work. Most of this work has been centered in Hawaii (102–106) and in France (13–25, 101, 118–123), but recently some interesting observations have come from Holland (100).

The work in Hawaii was directed against the root-knot eelworm (*Heterodera marioni*), and two methods were used. One of these was concerned with artificial stimulation into activity of natural enemies of the eelworm already present in the soil. It was found that when fresh plant material was incorporated into the soil, there was a rapid increase in the population of free-living nematodes. In one experiment, a 65-fold increase was obtained in 14 days, but, in spite of this, in 21 days the nematode population was much reduced and predacious fungi were very conspicuous. Apparently the increase in activity of the fungi overbalanced that of the eelworms. Tests showed that during such a period of increased activity the larvae of parasitic nematodes decreased in numbers. In a series of laboratory and glass-house tests, chopped pineapple plant material was mixed with naturally infected soil and allowed to decompose. In every experiment reductions in the numbers of infective *Heterodera* larvae were observed, and it was claimed that the results were statistically significant at the 0.1% level (102, 106).

Comparable results were obtained by application of other plant material, such as the grass *Panicum barbinode*, and it was suggested that this might explain the beneficial results that had been obtained by green manuring *Heterodera*-infested soils.

The second method used by the workers in Hawaii was the introduction of pure cultures of predacious hyphomycetes into soil infected with *Heterodera marioni* (105). In these experiments pineapple plants were grown in five-gallon pots of steamed soil, and three series of treatments were given. In one series nothing was added; in another the pots were infected with a known number of *Heterodera* larvae; while the third series received both larvae and an inoculation of one of six different predacious hyphomycetes. In all, the experiment comprised ten treatments, and each treatment was ten times replicated. The pots were kept under observation 15 months.

The results of the tests were disappointing. While a certain measure of control of the eelworm appeared to result from inocu-

lation with *Dactylella ellipsospora,* the other fungi used, consisting of *Arthrobotrys oligospora* (two isolates), *A. musiformis, Dactylella* sp. and *Dactylaria thaumasia,* were apparently without effect.

Much work has been done in France on the biological control of nematodes parasitic in plants (19, 25) and in domestic animals (13–16, 18, 24, 118, 119, 122, 123). In some early experiments on the eelworm causing pseudotuberculosis in horses (13) an ingenious method was devised for allowing eelworms to enter cultures of the fungi in as natural a manner as possible and with a minimum of contamination. Dung cultures containing the eelworms were covered with half Petri dishes containing agar cultures of *Arthrobotrys oligospora* and of *Dactylella bembicodes.* Pieces of filter paper were then arranged vertically in contact with the inner sides of the dishes containing the dung in such a manner that the nematodes were able to climb up them to gain entry to the fungus cultures. In this way it was possible to show that the nematodes were killed in large numbers by the fungi.

Other workers in France have demonstrated the ability of various predacious hyphomycetes to capture the larvae of eelworms parasitic in horses (118), cattle and sheep (14, 16, 18, 123), and even in the chimpanzee and in man (119). Little practical use appears to have been made of these discoveries. The fungi used were *Arthrobotrys oligospora, Dactylella ellipsospora* and *D. bembicodes.* The same three fungi were also shown to be capable of attacking successfully some of the nematodes causing disease in plants (19), and experiments on the actual control of plant pathogenic nematodes have been carried out with some success. In an attempt to control the root-knot eelworm (*Heterodera marioni*) in begonias, cultures of *Arthrobotrys oligospora* and of *Dactylella bembicodes* were added to infected compost in which the plants were growing, and it was found that the 50% infestation shown by the controls was reduced to 20% by *A. oligospora* and to 9% by *D. bembicodes* (25).

Some of the French workers have suggested methods for the preparation of fungal material in bulk, and in a form suitable for large-scale application to land. In one of these (21) the fungi were cultivated in a medium of oat straw, malt extract and agar, the spores being collected in due course, dried and distributed over the soil. In another method a liquid medium was used,

prepared from cooked maize and malt extract (24). When growth of the fungus was complete the liquid was drawn off, and the mycelium and its spores slowly dried, after which it was mixed with dry soil as a neutral medium to facilitate its distribution as a powder. I have tested the medium with *Arthrobotrys oligospora* and *A. robusta*, and have found both mycelium and spore production very good; I have not, however, tried its efficacy in the practical control of eelworms. Other methods of cultivation have also been suggested (17).

Other points arising in the pratical application of predacious fungi in nematode control have been investigated by the French school. One of these was the ability of predacious hyphomycetes to withstand lack of oxygen (23). It was thought that both *Arthrobotrys oligospora* and *Dactylella ellipsospora* were probably strict aerobes, since they grew only on the surface of liquid media, while *D. bembicodes* penetrated to a depth of 20 centimetres below the surface, suggesting a certain capacity for partial anaerobiosis. The possibility that the fungi might be potential parasites of cultivated plants was also examined, and no evidence of this could be observed when they were allowed to grow in contact with the vegetation in pastures; moreover, it was shown that animals grazing thereon took no harm from the fungi (20).

In view of some of the results obtained by the French workers, it is surprising that the possibility of using predacious fungi for the control of eelworms has not been more widely investigated, especially when one considers the immense damage to the crops of the world caused by these pests. Recently the matter has been taken up in Holland, and a description of a Pyrenomycete growing on the cysts of the potato root eelworm (*Heterodera rostochiensis*) has been described (100). During the last two years, unpublished work in my own Department has shown that the inoculation of soil with predacious hyphomycetes is a practical possibility, and that soil so inoculated retains the fungi for a reasonable period of time. Experiments have also indicated that the fungi are capable of reducing the population even of cyst-forming eelworms such as *Heterodera rostochiensis*. Much fundamental work remains to be done, however, before the value, if any, of biological control can be assessed accurately, and, as has been pointed out in an earlier communication (76), no useful

results in the practical field are likely to accrue until our knowledge of the biology and ecology of the predacious fungi has been greatly augmented.

TECHNIQUE

Many of the predacious fungi are not only very common but are also easy to grow in the laboratory. Their peculiar mode of life, however, makes them different from most fungi in some of their basic requirements, and in order to culture and observe them successfully some departures from conventional mycological technique are needed. A brief account of some of the methods that have been used in their study is presented here in the hope that it may be of help to those who wish to observe these interesting plants at first hand.

Mixed cultures of predacious fungi may readily be obtained by placing a small quantity of decaying plant material in the centre of a Petri dish containing sterile maize-meal agar. In order to prevent undue growth of moulds, the medium should be a weak one. A suitable medium may be prepared by adding 20 grams of maize-meal to rather more than a litre of tap water, and heating at 70° C. for one hour. The mixture is put aside to settle, and then a litre of clear supernatant liquid is decanted off, filtering through glass wool if necessary. To this, 20 grams of powdered agar are added, and the medium is autoclaved at 15 pounds per square inch for 20 minutes. Plates poured with this medium are suitable for nearly all predacious fungi.

After inoculation the plates are incubated at room temperature and observed periodically by examining the surface of the medium with a low power objective. Some of the more quickly growing predacious fungi may appear in about a week, while the slower ones, such as some of the hyphomycetes with constricting rings, may not be seen until two months or more after inoculation. The presence of predacious fungi can usually be recognised by the appearance of amoebae, somewhat rounded in shape, lying alongside very fine hyphae, or by dead or dying eelworms captured by predacious hyphomycetes. The characteristic conidia of both the Zoopagaceae and the *Arthrobotrys* series are often useful in detecting the presence of the fungi.

For examination of predacious fungi under higher powers of the microscope, preparations must be made. Living material is

far more satisfactory than stained specimens, and for its observation a small block of agar is cut out with a scalpel, the surface bearing the fungus being then sliced off as thinly as possible with a sharp razor blade and mounted in water, using a cavity slide so as to avoid crushing the specimen unduly when the cover glass is applied. If the specimen is to be preserved for more than an hour, the cover slip should be ringed with vaseline.

Permanent preparations of predacious fungi are not easy to make. The predacious hyphomycetes can be mounted quite satisfactorily in lactophenol coloured with a little cotton blue, but lactophenol is quite useless for most of the Zoopagaceae. Substitution of lactic acid for lactophenol allows fairly good preparations of Zoopagaceae to be made, but these do not last more than a few months.

For permanent mounts of Zoopagaceae, a method using chlorazol black is often useful, especially for showing the haustoria in amoebae. Thin slices of agar bearing the fungus are fixed in absolute alcohol for one minute, stained in a saturated solution of chlorazol black E in absolute methyl alcohol for ten minutes and mounted in Euparal, using a cavity slide. Full details of this and other methods have been given elsewhere (82, 83).

Excellent preparations of the predacious hyphomycetes of the *Arthrobotrys* series can be obtained by the Venetian turpentine method. From a block of agar the surface bearing the fungus is thinly sliced off and fixed in formalin-acetic acid (commercial formalin, 10 parts; glacial acetic acid, 5 parts; distilled water, 85 parts) for at least 24 hours. After washing until no smell of acetic acid remains, the slices are stained in Delafield's haematoxylin for a few minutes, differentiated in acid alcohol if necessary, "blued" in tap water or ammonia vapour, and placed in about five ml. of 10% glycerine in a watch glass, which is then left exposed to the air for the water to evaporate. When the glycerine has concentrated to a thick syrup, the dehydration is finished off in a desiccator. The process of concentration should take about two days. The material is then washed in absolute alcohol to remove the glycerine, and placed in a 10% solution of Venetian turpentine in absolute alcohol; this should be done in a desiccator, as the turpentine is extremely sensitive to moisture, and the material should be left in the desiccator until the turpen-

tine has thickened sufficiently for mounting. The agar slices are then mounted in an alcoholic solution of Venetian turpentine of suitable consistency, using a cavity slide.

The best preparations that I have yet obtained of the predacious hyphomycetes have been made by the following method. Blocks of agar bearing the fungus are fixed in formalin-acetic acid and washed. The block is then stuck, fungus downward, on a No. 1 cover slip by means of Szombathy's gelatine. The cover slip is left in a warm place—about 30° C.—until the agar begins to shrink, the face of the block bearing the fungus being prevented from shrinking because it is attached to the glass. At a stage in the drying process which can be determined only by experience the agar is cut away as close to the glass as possible, using a very sharp razor blade. The film of agar left on the cover slip contains the fungus; this can be stained with Heidenhain's haematoxylin, dehydrated, cleared in xylol and mounted in balsam. This method is not so difficult as it sounds, and gives superb preparations.

The endozoic hyphomycetes are difficult to handle, as the infected eelworms often succumb below the surface of the agar. Permanent preparations of these fungi are best made either in lactophenol or in glycerine jelly; for the latter technique, erythrosine is an excellent stain, and the preparations are improved if very slightly acidified with acetic acid, for this increases the brilliance of the stain.

Isolation of the *Arthrobotrys* series into pure culture presents few difficulties, since their conidia are carried on long erect fertile hyphae, and all that is needed is to pick off a spore or spores on the end of a sterile needle for transferring to a plate of sterile maize-meal agar. A very small block of sterile agar on the end of the needle helps a great deal in making the spores stick. Maize-meal agar is a good medium for pure cultures, and another that I have found useful is Langeron's potato-carrot-agar. Rabbit-dung-agar is another good one, particularly suitable for *Trichothecium flagrans,* which is a difficult species to maintain in pure culture because it so readily loses its power of sporulation. Rich media should not be used for culturing predacious hyphomycetes, and enrichment of media with added carbohydrate should be avoided. Malt agar and, especially, potato-dextrose-agar are, in my opinion, thoroughly bad for predacious fungi.

Pure cultures of predacious hyphomycetes usually do not show the characteristic traps, and to induce trap formation nematodes must be offered to the fungi. This is best done by transferring the fungus to an agar plate already infested with nematodes, rather than by adding nematodes to a flourishing culture of the fungus. One of the major difficulties in working with predacious fungi is maintenance of adequate stocks of eelworms for use when they are required.

CONCLUSION

From the foregoing account it will be seen that our knowledge of the predacious fungi has many gaps. On the morphological side a great deal of work has been done, but their physiology and ecology are virtually unexplored, and many fascinating lines of research are here waiting for investigation. Many taxonomic problems also need clearing up.

The nematode-attacking fungi have a particular economic interest at the present time, for the losses in agricultural produce caused by nematodes are enormous and attempts to control them by chemical means have so far done little more than underline the gravity of the situation. Work already done has suggested that the predacious fungi may be capable of reducing eelworm populations on a useful scale if practical means for their application can be found, but a great deal of investigation is needed before an accurate estimate of their value, if any, can be made. If the predacious fungi are to provide even a partial solution to the eelworm problem, research on a large scale over a period of years will have to be undertaken, needing the financial assistance that only governments can provide. It must be emphasised that their premature use against eelworms in the field is likely to do more harm than good; if success is to be achieved the work must be founded on a knowledge of the ecology and biology of the fungi that can come only from fundamental research.

SUMMARY

The predacious fungi include members of all the Classes but are mainly contained in two groups: the Zoopagaceae and the hyphomycetes. A few have been known for a long time, but most have been discovered during the last two decades. Some predacious fungi were known as "saprophytes" for many years before their true nature was realised. The animals attacked by these

fungi are mostly amoebae and nematode worms, but rotifers and other microscopic animals are also included among their prey. The animals may be trapped by some mechanism such as sticky hyphae or mechanically constricting rings, or there may be adhesive spores that stick to the exterior of the host.

Among the Zoopagaceae, a Family of the Zygomycetes, we find both endozoic and animal-trapping fungi. The endozoic species mainly attack Protozoa, the spores either sticking to the host as it moves about or being ingested. The animal-trapping forms usually consume amoebae, but some of the larger ones capture eelworms which are caught by a sticky secretion of the mycelium. The contents of the captured animal are absorbed by means of haustoria. The Zoopagaceae reproduce asexually by true conidia; where the sexual stage is known, it involves fusion of equal or unequal gametangia.

The *Arthrobotrys* series of predacious hyphomycetes capture their prey, usually nematode worms, by some kind of trap. The traps may be sticky hyphal loops and networks, sticky knobs or branches, hyphal rings into which the eelworms are wedged by their own efforts to pass through, or similar rings in which the cells are sensitive to contact stimuli and are able to swell so as to hold the eelworm by constriction. After the captured animal is dead, its body contents are absorbed by trophic hyphae which fill its carcass.

The endozoic predacious hyphomycetes are parasitic within the bodies of eelworms. The animal is usually infected by means of a sticky spore that becomes attached to its integument. The internal mycelium grows at the expense of the host until the animal is completely filled with hyphae, after which fertile branches from the mycelium pass out through the integument of the dead host into the air and produce their conidia. These endozoic hyphomycetes are taxonomically a mixed group; one genus appears to be an imperfect basidiomycete.

In addition to these two main groups of predacious fungi, others are known. A number of species belong to the Lagenidiales and are parasitic in eelworms. *Zoophagus insidians,* predacious on rotifers, was one of the first predacious fungi to be described; it was placed in the Saprolegniales but may possibly belong to the Zoopagaceae.

The working of the nematode traps of *Arthrobotrys* and its

allies has been investigated by a number of workers. The traps are usually formed only in the presence of eelworms. Sticky traps are adhesive only for eelworms unless they are exposed to dry air, while the constricting ring trap can be sprung by rubbing the inside of the ring with a glass micromanipulator needle. The actual mechanism by which the swelling of the ring is brought about has not been elucidated. In other respects, the physiology of the predacious fungi is almost a closed book.

Little is known about the ecology of predacious fungi. They have been obtained from a variety of sources such as leaf-mould, dung, soil, decaying plant remains, water, rotting wood and living bryophytes, but our knowledge of them is insufficient to allow of any ecological generalisations, and quantitative information is badly needed.

The predacious fungi vary in their degree of host-specificity. The Zoopagaceae that attack Protozoa are usually limited to a single species for prey, but those capturing eelworms are more versatile. The nematode-trapping hyphomycetes usually capture a wide range of species, the limit being mainly one of size, though the structure of the cuticle of the nematode may also be of importance. The endozoic hyphomycetes sometimes show some degree of host-specificity, but are variable in this respect.

The means of dispersal in the predacious fungi is unknown, though it is suggested that their spores might be carried by air currents, small arthropods, earthworms and, in some instances, water. The endozoic hyphomycetes are probably dispersed by their host animals.

Attempts have been made to use the predacious hyphomycetes for the biological control of eelworms. Methods of producing them in quantity have been worked out, and experiments in their actual use have met with a certain amount of success on a small scale. Further work on this may produce interesting results.

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